# THE EPIZOOTIOLOGY AND TRANSMISSION OF *EUSTRONGYLIDES IGNOTUS* (DIOCTOPHYMATOIDEA) IN INTERMEDIATE HOSTS IN FLORIDA

By

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TABLE OF CONTENTS

page

ACKNOWLEDGMENTS . . . . . iii

LIST OF TABLES . . . . . ix

LIST OF FIGURES . . . . . xii

ABSTRACT . . . . . xv

INTRODUCTION . . . . . 1

Background of the Problem . . . . . 1
General . . . . . 2
Objectives . . . . . 11

METHODS AND MATERIALS . . . . . 13

Study Sites . . . . . 13
Collection and Examination of Fishes, Amphibians, and Reptiles . . . . . 17
Fish Density . . . . . 19
Collection and Examination of Oligochaetes . . . . 20
Collection and Analysis of Water Data . . . . . 21
Collection and Analysis of Sediment Data . . . . . 22
Collection and Analysis of Vegetation Data . . . . . 23
Collection and Culture of Eggs of *E. ignotus* . . . . 24
Collection, Maintenance, and Experimental Infection of Oligochaetes . . . . . 26
Collection, Maintenance, and Experimental Infection of Fishes . . . . . 27
Fish Behavior Study . . . . . 29
Statistical Analysis . . . . . 31

RESULTS . . . . . 33

Statewide Survey of Fishes . . . . . 33
Distribution of Infected Fish . . . . . 33
Species of Fish Collected . . . . . 36
Size and Gender of Infected Mosquitofish . . . . . 40
Seasonal Infections in Fishes . . . . . 43
Fish Densities . . . . . 44
Amphibians and Reptiles . . . . . 47
Results of Oligochaete Survey . . . . . 47
Physico-chemical Characteristics of Water . . . . . 55
Physico-chemical Characteristics of Sediment . . . . 62
Sediment Grain Size . . . . . 65
Results of Vegetation Survey . . . . . 68
Results of Lake Alice Study . . . . . 72
Fish Survey . . . . . 72
Oligochaete Survey . . . . . 78
Physico-chemical Characteristics of Water . . . 80
Physico-chemical Characteristics of Sediment . 85
Sediment Grain Size . . . . . 90
Vegetation Survey . . . . . 90
Laboratory Results . . . . . 94
Morphology and Development of Eggs . . . . . 94
Development of Eggs at Various Temperatures . 101
Development of Eggs - Saline Conditions . . . 102
Development of Eggs - Drydown Conditions . . 104
Experimental Infection of Oligochaetes and Chironomids . . . . . 105
Experimental Infection of Oligochaetes at Various Temperatures . . . . . 107
Second-stage Larvae (L-2) in Oligochaetes . . 109
Third-stage Larvae (L-3) in Oligochaetes . . 113
Experimental Infection of Fishes . . . . . 117
Transmission from infected oligochaetes (L-2) . . . . . 117
Transmission from infected oligochaetes (L-3) . . . . . 117
Direct transmission from eggs . . . . . 120
Major dimensions of third-stage larvae from fish . . . . . 126

Major dimensions of fourth-stage larvae from fish . . . . . 130
Experimental transmission to paratenic hosts (fish) . . . . . 137
Behavioral Changes in Infected Fishes . . . . 145

DISCUSSION . . . . . 151

Distribution . . . . . 151
Fishes . . . . . 158
Oligochaetes . . . . . 162
Physico-chemical Characteristics of Water and Sediment . . . . . 166
Vegetation . . . . . 176
Lake Alice . . . . . 179
Life Cycle . . . . . 184
Eggs . . . . . 184
*Eustrongylides ignotus* in Oligochaetes . . . 187
*Eustrongylides ignotus* in Fishes . . . . . 193
*Eustrongylides ignotus* in Paratenic and Abnormal Hosts . . . . . 199
Fish Behavior . . . . . 202
Life Cycle Summary . . . . . 207
Management Implications . . . . . 211

CONCLUSIONS . . . . . 214

APPENDIX . . . . . 220

LITERATURE CITED . . . . . 230

BIOGRAPHICAL SKETCH . . . . . 245

LIST OF TABLES


| Table | | page |
|---|---|---|
| 1. | Regional comparison of study site types | 34 |
| 2. | Fishes collected and examined for larvae of *Eustrongylides ignotus* | 37 |
| 3. | Fishes collected from positive sites | 39 |
| 4. | Amphibians and reptiles examined for larvae of *Eustrongylides ignotus* | 48 |
| 5. | Aquatic oligochaetes collected from study sites | 49 |
| 6. | Comparison of common aquatic oligochaetes collected from study sites | 53 |
| 7. | Physico-chemical characteristics of water collected during wet season 1995 | 56 |
| 8. | Physico-chemical characteristics of water collected during dry season 1995 | 57 |
| 9. | Physico-chemical characteristics of water collected during wet season 1996 | 58 |
| 10. | Physico-chemical characteristics of water collected during dry season 1996 | 59 |
| 11. | Results of analysis of sediment samples collected during the wet season 1995 | 63 |
| 12. | Results of analysis of sediment samples collected during the dry season 1995 | 63 |

13. Results of analysis of sediment samples collected during the wet season 1996 . . . . . 64

14. Results of analysis of sediment samples collected during the dry season 1997 . . . . . 64

15. Fishes examined from Lake Alice . . . . . 73

16. Oligochaetes examined from Lake Alice . . . . . 79

17. Physico-chemical characteristics of water collected from Lake Alice . . . . . 81

18. Physico-chemical characteristics of sediment collected from Lake Alice . . . . . 87

19. Sediment grain size at Lake Alice . . . . . 91

20. Vegetation at Lake Alice . . . . . 92

21. Development of eggs at various temperatures . . . . 102

22. Development of eggs at various saline concentrations . . . . . 104

23. Development of eggs under drydown conditions . . . 105

24. Number of infected oligochaetes at 28 C . . . . . 108

25. Number of infected oligochaetes at 19.5 C . . . . . 108

26. Major dimensions of third stage larvae from infected oligochaetes . . . . . 116

27. Number of mosquitofish (*Gambusia holbrooki*) fed infected oligochaetes . . . . . 119

28. Number of mosquitofish (*Gambusia holbrooki*) fed infective eggs . . . . . 124

29. Number and gender of infected mosquitofish (*Gambusia holbrooki*) fed infective eggs . . . . . 125

30. Number of mosquitofish (*Gambusia holbrooki*) fed infected oligochaetes . . . . . 129

31. Major dimensions of third stage larvae from fish fed eggs . . . . . 130

32. Major dimensions of fourth stage larvae from naturally infected fish . . . . . 135

33. Major dimensions of fourth stage larvae from fish fed eggs . . . . . 136

34. Number and location of fourth stage larvae from paratenic hosts . . . . . 143

35. Number of times infected and non-infected prey selected first, mean number of capture attempts, and mean time until capture . . . . . 147

36. Number and location of larvae recovered from experimental predators . . . . . 149

LIST OF FIGURES


| Figure | | page |
|---|---|---|
| 1 | Map of study sites in Florida | 14 |
| 2 | Map of study sites in Lake Alice watershed | 16 |
| 3 | Behavior experiment apparatus | 30 |
| 4 | Mean length and depth of mosquitofish | 41 |
| 5 | Seasonal comparison of infected mosquitofish | 43 |
| 6 | Mean density of fish collected during dry season | 45 |
| 7 | Mean density of fish collected during wet season | 46 |
| 8 | Mean density of oligochaetes | 54 |
| 9 | Mean percent sediment particle size | 66 |
| 10 | Percent composition of sediment | 67 |
| 11 | Mean percent vegetation composition during wet season | 69 |
| 12 | Mean percent vegetation composition during dry season | 70 |
| 13 | Prevalence of infected fishes at Lake Alice | 75 |
| 14 | Density of fishes at Lake Alice | 77 |
| 15 | Dissolved oxygen concentrations at Lake Alice | 83 |
| 16 | Total nitrogen concentrations at Lake Alice | 84 |

Figure page

17 Chlorophyll-a concentrations at Lake Alice . . . . . 86

18 Sediment total carbon concentrations at Lake Alice . . . . . 88

19 Sediment total phosphorus concentrations at Lake Alice . . . . . 89

20 Scanning electron micrograph of egg of *Eustrongylides ignotus* . . . . . 96

21 Scanning electron micrograph of eggshell of *Eustrongylides ignotus* . . . . . 97

22 Egg of *Eustrongylides ignotus* at 1-day . . . . . 98

23 Egg of *Eustrongylides ignotus* at 5-days . . . . . 99

24 Egg of *Eustrongylides ignotus* at 21-days . . . . . 100

25 First-stage larva of *Eustrongylides ignotus* . . . 103

26 Scanning electron micrograph of cephalic extremity of second-stage larva of *Eustrongylides ignotus* . . . . . 110

27 Scanning electron micrograph of caudal extremity of second-stage larva of *Eustrongylides ignotus* . . . . . 111

28 *Limnodrilus hoffmeisteri* with second-stage larva of *Eustrongylides ignotus* . . . . . 112

29 Histologic sagittal section of *Limnodrilus hoffmeisteri* with second-stage larva of *Eustrongylides ignotus* . . . . . 114

30 *Gambusia holbrooki* with cyst containing larva of *Eustrongylides ignotus* . . . . . 122

31 *Gambusia holbrooki* with fourth-stage larva of *Eustrongylides ignotus* . . . . . 123

Figure page

32 Scanning electron micrograph of cephalic extremity of third-stage larva of *Eustrongylides ignotus* . . . . . 127

33 Scanning electron micrograph of caudal extremity of third-stage larva of *Eustrongylides ignotus* . . . . . 128

34 Cephalic extremity of male fourth-stage larva of *Eustrongylides ignotus* . . . . . 131

35 Caudal extremity of male fourth-stage larva of *Eustrongylides ignotus* . . . . . 132

36 Cephalic extremity of female fourth-stage larva of *Eustrongylides ignotus* . . . . . 133

37 Caudal extremity of female fourth-stage larva of *Eustrongylides ignotus* . . . . . 134

38 Scanning electron micrograph of cephalic extremity of fourth-stage larva of *Eustrongylides ignotus* . . . . . 138

39 Scanning electron micrograph of cephalic papillae of fourth-stage larva of *Eustrongylides ignotus* . . . . . 139

40 Scanning electron micrograph of inner circle papilla of fourth-stage larva of *Eustrongylides ignotus* . . . . . 140

41 Scanning electron micrograph of caudal extremity of fourth-stage larva of *Eustrongylides ignotus* . . . . . 141

42 Histologic cross section of *Gambusia holbrooki* . 142

43 *Pomoxis nigromaculatus* with fourth-stage larva of *Eustrongylides ignotus* . . . . . 143

44 Life cycle of *Eustrongylides ignotus* . . . . . 208

Abstract of Dissertation Presented to the Graduate School of the University of Florida in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

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December 1998

Chairperson: Donald J. Forrester
Major Department: Wildlife Ecology and Conservation

Between August 1994 and October 1997 field and laboratory studies were conducted to examine the life cycle of the wading bird parasite *Eustrongylides ignotus*. A total of 63,819 fishes, amphibians, and reptiles was collected from 176 sites, throughout peninsular Florida and examined for larval *E. ignotus*. Infected fishes were identified from 30 (17%) sites. Based on prevalences, mosquitofish (*Gambusia holbrooki*) and sunfish(Centrarchidae) were the

most important intermediate hosts. No infected amphibians or reptiles were collected. A total of 10,508 oligochaetes was identified. Immature tubificids without hair setae, *Dero digitata*, and *L. hoffmeisteri* were the most abundant oligochaetes at positive sites. Mean abundances of fishes and oligochaetes were higher at positive than at negative sites.

Signs of frequent human disturbance, such as removal of sediment, were observed at all positive sites. In addition, all positive sites had a history of receiving anthropogenic nutrient input such as sewage effluent, urban runoff, or agricultural runoff. All unaltered natural sites were negative. In the surface water, dissolved oxygen was lower, while total nitrogen, total phosphorus (TP), and chlorophyll-a were higher at positive than negative sites. In sediment, soil oxygen demand, TP, and mean grain size were higher at positive sites. Macrophytes at positive sites were characterized by higher percent emergent vegetation and grasses than at negative sites. Under laboratory conditions, mosquitofish became infected after ingestion of parasite eggs or aquatic oligochaetes containing *Eustrongylides* larvae. Total time for completion

of the life cycle was 115 to 150 days when fish ingested eggs and 338 to 408 days when fish ingested infected oligochaetes. Four species of large predatory fishes, including bluegill (*Lepomis macrochirus*), crappie (*Pomoxis nigromaculatus*), largemouth bass (*Micropterus salmoides*), and warmouth (*Lepomis gulosus*) were fed mosquitofish containing *Eustrongylides* larvae. At necropsy, live parasite larvae were recovered from all predators. Several predators had multiple infections after ingesting > one larva, indicating that bio-amplification of the parasite in the food chain does occur. In the laboratory, infected mosquitofish were more susceptible to predation by centrarchids than were uninfected mosquitofish. Infected mosquitofish were selected first and required fewer capture attempts than uninfected mosquitofish. In addition, aberrant behavior including lethargy, convulsions, and buoyancy abnormalities were observed in 67% of predators after ingesting infected prey. Because of aberrant behaviors, these hosts may be more susceptible to predation by wading birds than uninfected fish.

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# INTRODUCTION

## Background of the Problem

In Florida, the number of reproducing wading birds (Ciconiiformes) has declined dramatically over the past 40 years (Kushlan and White 1977, Ogden 1978, Ogden 1994). Several reasons for this decline have been proposed, including habitat alterations, emigration, and diseases (Frederick and Spalding 1994). Frederick and Collopy (1988) suggested that anthropogenic habitat degradation, losses of major feeding areas, and disruption of normal hydropatterns were important factors in the decline of these populations. The authors suggested also that repeated reproductive failure at historic breeding colonies in Florida may have resulted in emigration of breeding populations to other areas in the southeastern USA. Spalding et al. (1993) reported that infection with the parasite *Eustrongylides ignotus* was a major mortality factor in nestling wading birds in Florida, causing losses of up to 80% in some colonies. Repeated reproductive failure, as a result of

this disease, could be a factor in the decline of breeding wading birds in Florida.

*Eustrongylides ignotus* is a robust parasitic nematode whose definitive host is the pisciverous bird, especially ardeids (Spalding and Forrester 1993). The nematode perforates the stomach of the host and migrates into the surrounding tissue, maintaining an opening into the gastrointestinal tract through which eggs are deposited. The parasite may tunnel through major organs, such as the liver, spleen, gonads, and intestines, resulting in permanent damage. Hemorrhage, secondary bacterial infections, and loss of appetite may occur as a result of these lesions and can result in morbidity or mortality, and changes in growth rate especially in nestling ciconiiforms (Spalding and Forrester 1993, Spalding et al. 1994).

## General

*Eustrongylides* spp. have been reported from 8 orders of birds worldwide, including Anseriformes, Ciconiiformes, Charadriiformes, Falconiformes, Gaviiformes, Passeriformes, Pelecaniformes, and Podicipediformes (Shillinger 1936, Bowdish 1948, Locke 1961, Locke et al. 1964, Winterfield and Kazacos 1977, Karmanova 1968, Measures 1988*a*, and Spalding

et al. 1993). The genus is cosmopolitan in distribution, probably the result of this broad range of susceptible and mobile hosts.

Three valid species of *Eustrongylides* and their distributions were proposed in the redescription of the genus by Measures (1988a). These include *E. excisus* Jägerskiöld, 1909; *E. tubifex* Jägerskiöld 1909; and *E. ignotus* Jägerskiöld 1909. Distinguishing characteristics include size and arrangement of cephalic papillae and morphology of the caudal sucker in males (Karmanova 1968, Measures 1988a). The cephalic extremities of all species of *Eustrongylides* have 12 labial papillae arranged in 2 concentric circles around the mouth. The inner circle papillae of *E. excisus* are larger than those of the outer circle, which distinguishes this species from *E. tubifex*. In addition, the caudal sucker of *E. excisus* is characterized by a deep ventral cleft, which distinguishes it from both *E. tubifex* and *E. ignotus*. The inner circle cephalic papillae of *E. tubifex* are smaller than those of the outer circle, distinguishing this parasite from other *Eustrongylides* spp. In addition, the outer perimeter of the caudal sucker of *E. tubifex* has a reduced cuticular hem.

The distinguishing characteristics of *E. ignotus* include inner circle papillae larger than those of the outer circle, the outer perimeter of the caudal sucker has a wide cuticular hem, and the caudal sucker lacks a ventral cleft. Although absent from the New World, *E. excisus* has the widest known distribution and has been reported from Europe, Russia, Middle East, India, Taiwan, and Australia. The distribution of *E. tubifex* includes Europe, Russia, and the Holartic and Neotropical regions of the New World. *Eustrongylides ignotus* has been reported from the Nearartic, Neotropical, and Australian regions.

In the North America, only *E. tubifex* and *E. ignotus* are recognized currently (Measures 1988a). The distribution of *E. tubifex* appears to be limited to the northern latitudes of the USA (the Great Lakes region primarily) and Canada, while *E. ignotus* is distributed widely throughout the temperate and subtropical regions of North America and is the only species reported from Florida. The importance of these 2 species, as a cause of morbidity and mortality of piscivorous birds, appears to differ significantly. Mergansers are probably the most important host of *E. tubifex* (Measures 1988a), but few reports exist of large-

scale host mortality, associated with infection with this parasite (Sprinkle 1973). Under experimental conditions, Measures (1988a) reported that *E. tubifex* matured rapidly in the proventriculus of common mergansers (*Mergus merganser*), eliciting raised oval tumors which resolved to normal condition approximately 30-days post-infection. In contrast, ciconiiforms are the most important host of *E. ignotus*. Within this group, ardeids are the only reported definitive hosts and epizootics of eustrongylidosis in herons and egrets have been reported from California, Delaware, Louisiana, Texas, and Florida (Weise et al. 1977, Roffe 1988, Spalding et al. 1993, Franson and Custer 1994). *Eustrongylides ignotus* is especially pathogenic to nestling ardeids, with mortalities of up to 80% reported from colonies in Delaware and Florida (Weise et al. 1977, Spalding et al. 1993). Under experimental conditions, Spalding and Forrester (1993) reported that migrating *E. ignotus* larvae caused hemorrhage and bacterial peritonitis in nestling ardeids, which often resulted in death of the host. The authors suggested that nestling ciconiiforms were not normal hosts for *E. ignotus*, as death of nestlings usually occurred before infections became patent.

Fish are important intermediate hosts of all *Eustrongylides* spp. and piscivorous birds become infected after consuming fourth-stage larvae in fish. *Eustrongylides* larvae have been reported from 17 orders of fishes worldwide, including Acipenseriformes, Amiiformes, Anguilliformes, Antheriniformes, Beryciformes, Clupeiformes, Cypriniformes, Gadiformes, Gastorosteiformes, Perciformes, Petromyziformes, Pleuroformes, Salmoniformes, Semionotiformes, Siluriformes, Symbranchiformes, and Tetrodontiformes (Bangham 1940, Karmanova 1968, Spalding et al. 1993). It is clear from the broad range of susceptible intermediate hosts that *Eustrongylides* spp. are not restricted to fishes of any particular taxonomic group or fishes that have specialized types of feeding behavior. Karmanova (1968) infected great cormorants (*Phalacrocorax carbo*) with fourth-stage larvae of *E. excisus* from wild fish collected from the Volga River (Russia). Measures (1988*b*) infected 6 species of birds including common mergansers, red-breasted mergansers (*M. serrator*), hooded mergansers (*M. cucullatus*), great blue herons (*Ardea herodias*), double-crested cormorants (*P. auritus*), and domestic ducks (*Anas platyrhynchos*) with *E. tubifex* collected from naturally

infected fish from Guelph Lake and Lake Erie, Canada. Spalding and Forrester (1993) infected tricolored herons (*Egretta tricolor*) and great white herons (*Ardea herodius occidentalis*) with *E. ignotus* from naturally infected fish from Florida.

A variety of piscivorous poikilotherms may function as paratenic hosts also. These include fishes (Karmanova 1968, Measures 1988*d*), amphibians (Panesar and Beaver 1979), and reptiles (Lichtenfels and Lavies 1976, Goldberg et al. 1991). Cooper et al. (1978a,b) transferred *E. tubifex* larvae from fish to predatory yellow perch (*Perca flavescens*), grass frogs (*Rana pipiens*), snapping turtles (*Chelydra serpentina*), Blanding's turtle (*Emys blandingi*), and spiny soft-shelled turtle (*Trionyx spinifer*). Measures (1988*d*) infected pumpkinseed (*L. gibbosus*) paratenic hosts with encapsulated fourth-stage *E. tubifex* larvae from naturally infected pumpkinseed, rock bass (*Ambloplites rupestris*), and yellow perch (*Perca flavescens*). Although advanced-stage *Eustrongylides* larvae, similar to those reported from fish, have been observed in several species of amphibians and reptiles, experimental transmission from these animals to birds was not done. Because of the

similarity of morphology and behavior of larvae in fish, amphibians, and reptiles, fourth-stage larvae in all poikilotherms are probably infective to birds.

After consumption by the bird, the parasite penetrates the stomach wall, migrates into surrounding tissue, matures to the adult-stage, and mates. An opening is maintained into the stomach, through which parasite eggs are passed. The eggs are excreted into the environment with feces.

The remainder of the life cycle is less clear. In fact, researchers have reported difficulty infecting fish intermediate hosts with *Eustrongylides* under experimental conditions (Measures 1988*d*). Although the life cycles of *E. excisus* and *E. tubifex* have been examined, information on the life history of *E. ignotus* is incomplete (Karmanova 1968, Measures 1988*b,c,d*). All members of the Dioctophymidea, whose life histories are known, utilize an annelid as the first intermediate host (Mace and Anderson 1975) and it was proposed, but not proven experimentally, that aquatic oligochaetes were the first intermediate host of all *Eustrongylides* spp. (Karmanova 1968). Karmanova (1968) reported that *E. excisus* developed to the third larval stage in *Lumbricus variegatus*, *Tubifex tubifex*, and

*Limnodrilus* sp., although it was unclear if experimental transmission to fish was successful. Measures (1988*c*) infected the aquatic oligochaetes *L. hoffmeisteri* and *T. tubifex* with *E. tubifex*, but was unable to transfer the parasite from oligochaetes to fish experimentally. Prior to the present study, attempts at experimental infection of oligochaetes with *E. ignotus* was not successful (M. G. Spalding, pers. com.). Thus, the life cycle of this parasite is uncertain and may include 2 intermediate hosts (oligochaetes and fish) and a variety of paratenic hosts (piscivorous fish, amphibians, and reptiles).

Many researchers have suggested that outbreaks of eustrongylidosis in piscivorous birds were associated with anthropogenic alterations of aquatic systems that resulted in increased numbers of potential intermediate hosts (Measures 1988*b*, Spalding et al. 1993, Franson and Custer 1994, Frederick et al. 1996). To date, little information is available on the environmental conditions associated with these outbreaks. Organic enrichment of surface water and sediment was associated with epizootics of eustrongylidosis in the Chesapeake Bay area by Hirshfield et al. (1983), but chemical studies on water and sediment were not done. In

Florida, Spalding et al. (1993) reported that disturbed soil, exogenous nutrients, and high densities of oligochaetes were important contributing factors to the prevalence and distribution of *E. ignotus* in fish. Frederick et al. (1996) reported that the prevalence and distribution of the parasite in fishes in Florida were much greater today than in the past and attributed this to increased exogenous nutrient input from expanding human habitation.

Although many aquatic systems in Florida were oligotrophic in the past (Canfield 1981), anthropogenic nutrient input has altered some of these to eutrophic systems and the resulting changes of flora and fauna may have resulted in increased numbers of potential intermediate hosts (Pezeshki 1987). If this is true, wading birds in Florida may be at greater risk of eustrongylidosis today than in the past.

In this study, I attempted to answer some of the fundamental questions about the life cycle of *E. ignotus* in Florida and to suggest management practices that may help to minimize the risk of eustrongylidosis in wading birds.

## Objectives

The goal of this study was to understand the life cycle of *Eustrongylides ignotus* and to provide information to managers which may effectively limit the risk of this wildlife disease problem. In order to accomplish this, the following objectives were established:

1. Determine which oligochaete worms, or other aquatic invertebrates, are primary intermediate hosts for the parasite.
2. Determine which fish in Florida serve as intermediate hosts.
3. Determine the distribution in Florida of intermediate hosts (fishes and oligochaetes) infected with larvae of *Eustrongylides*.
4. Determine the environmental conditions necessary for the production of large numbers of primary intermediate hosts (oligochaetes) and infected secondary intermediate hosts (fish).

5. Determine how long parasite eggs take to become infectious to oligochaete worms after being excreted by birds.

6. Determine the time it takes for the parasite to mature to the infective stage in fishes, once ingested.

7. Determine the role of other organisms, such as turtles, frogs, and snakes as possible secondary intermediate hosts at sites with infected fish.

8. Determine behavioral changes in infected fish.

9. Synthesize the results of the above objectives into guidelines for the management of surface waters so as to minimize the proliferation of the parasite.

## METHODS AND MATERIALS

### Study Sites

Between August 1994 and October 1997 a survey of 176 wading bird foraging areas was conducted throughout peninsular Florida. Potential intermediate hosts of *Eustrongylides ignotus* (fishes, amphibians, and reptiles) were collected and examined from each of these sites. Sites where infected intermediate hosts were collected were designated "Positive", while those with no infected intermediate hosts were "Negative". A total of 36 sites in north-central Florida (28° 55' to 30° 00' N, 81° 45' to 82° 30' W), 33 sites in central Florida (27° 30' to 28° 54' N, 81° 35' to 82° 25' W), and 107 sites in southern Florida (25° 10' to 27° 29' N, 80° 10' to 81° 03' W) was selected (Fig. 1). Site descriptions and locations are given in Appendix A. Site selections were based on: 1) wading birds observed foraging at the site, 2) wading bird tracks were

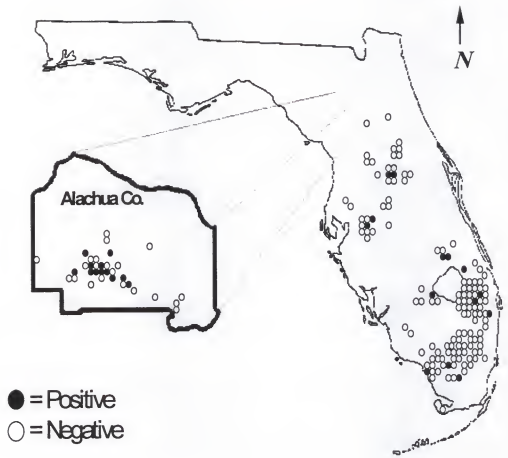


Figure 1. Map of Florida with distribution of study sites, 1994-1997. Sites with *Eustrongylides ignotus* larvae in fish are shown with solid circles. Negative sites are shown with open circles.

visible at the shoreline and in the littoral zone, or 3) moribund birds, infected with *E. ignotus*, were recovered near the site. Both natural and human-altered sites were selected. Sites were separated into categories of Natural (N), Urban (U), Agricultural (A), and Sewage (S). Natural sites included pristine lakes, ponds, streams, and marshes. Sites with human alterations included stormwater runoff retention ponds and streams, canals, waters adjacent to boat launching ramps, dairy waste retention ponds, agriculture runoff areas, and human sewage outflow systems. Although some of the sites may have received surface water from a combination of sources, major input sources were generally obvious and sites were classified as such. Most sites were accessible by driving or walking, but some sites (in southern Florida primarily) were accessible by airboat only. Water depths ranged from 0.25 to 1.5 M.

The Lake Alice watershed (Alachua County, N 29° 38.4', W 82° 20.8') was divided into 4 study sites (Fig. 2). These sites were sampled quarterly for water and sediment analysis, fish, and oligochaetes.

The Lake Alice study sites were selected because larvae of *Eustrongylides* in fish were collected near the

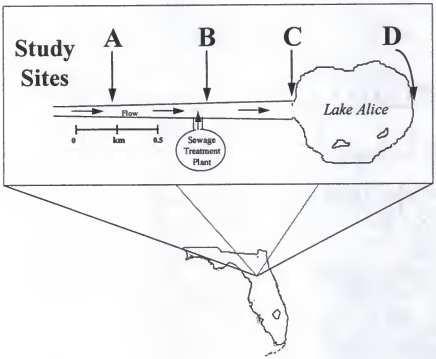


Figure 2. Map of study sites in Lake Alice watershed (Alachua County, N 29° 38.4', W 82° 20.8') Florida, 1994 - 1997.

sewage treatment plant in 1990 by Spalding et al. (1993). Lake Alice received treated human effluent since 1926, when the first sewage treatment plant was constructed (Korhnak 1996). Prior to 1926, raw sewage was discharged into the marsh surrounding Lake Alice for several years. In 1947 a sewage treatment plant was constructed and wastewater was discharged into a nearby sinkhole. The sinkhole was sealed off in 1964 and, for the next 30-yrs, effluent was discharged directly into the Lake Alice watershed. An advanced wastewater treatment plant was completed in October 1994 and began operation on November 18, 1994. Treated effluent was diverted from the Lake Alice watershed in November 1994 and piped directly to injection wells, with additional wastewater used for irrigation of a municipal golf course.

Collection and Examination of Fishes, Amphibians, and Reptiles

All animal collection methods and techniques were approved by the Institutional Animal Care and Use Committee (I.A.U.C.), Univ. of Fla. Fishes ($n$ = 63,415), amphibians ($n$ = 334), and reptiles ($n$ = 34) were collected using seine-net, dip-net, minnow trap, electro-shock, and hook and line

techniques. Some amphibians were captured at night, using dip-nets and spotlights. Specimens were anesthetized on ice, killed using MS-222® (tricaine methanesulfonate) (Syndel Laboratories Ltd., 8879 Selkirk St., Vancouver, B.C., Can.), decapitation or cervical dislocation, and transported to the laboratory on ice. Samples were either examined immediately or frozen and examined as soon as possible. Specimens were identified to species and standard length (SL) and body depth were recorded. Large fish were decapitated and the body cavity was opened from the pelvic fins to the anus. Small fish were eviscerated using a scalpel and surgical probes. The viscera were removed and examined under a variable power (7 to 30 x) dissecting microscope for the presence of *Eustrongylides* larvae. The coelomic cavity was examined and a sagittal section made of each fish. All muscle tissues were then sectioned and observed under the microscope. Amphibians and reptiles were dissected using standard techniques and all organs and major muscle tissues were examined. The number, location, and size of all *Eustrongylides* larvae were recorded. Most worms were preserved in 70% glycerin alcohol (1 part glycerin: 9

parts 70% ethyl alcohol). Some larvae were cleared in lactophenol and studied under the light microscope.

Ecological terms in parasitology followed Margolis et al. (1982). Prevalence was expressed as a percentage of the number of individuals of a host species infected with the parasite ÷ the number of hosts examined. Mean intensity was expressed as the total number individual parasites of the same species in a sample of hosts ÷ the total number of infected individual hosts in the sample.

Fish Density

At selected study sites (Appendix B), a relative index of fish density was calculated following Ricker (1975). Seine nets only were used for these calculations and fish were retained for laboratory examination. The area of seine net deployed, linear distance of water seined (in meters), and number of fish collected were recorded. Relative density was calculated using:

$$D = N / A$$

Where:

D = Density (No. Fish / $m^3$)

N = No. fish collected

A = Area [Seine net length (m) x seine net depth (m) x linear distance covered (m)]

Collection and Examination of Oligochaetes

Oligochaete specimens were obtained following Brinkhurst (1986). Specimens were collected using a 8.9 (I.D.) x 12.7cm PVC corer. Samples were collected over a 3-yr period (1994 - 1997) from positive ($n$ = 22) and negative sites ($n$ = 36) on a quarterly basis to account for seasonal variation in total numbers and species composition. Site descriptions and locations are given in Appendix B. A relative index of oligochaete abundance was determined by counting and recording the number of oligochaetes in each core (579 $cm^3$). Samples were screened through a 300 $\mu$m pore mesh, fixed in 10% formalin, and stained with Rose Bengal (Eastman Kodak Co., Rochester, NY). Samples were transported to the laboratory in plastic bags, transferred to 1 L glass jars and allowed to settle for 24-hr. Formalin was then decanted and the samples preserved in 70%

alcohol. Oligochaetes were counted, mounted and cleared on glass slides using CMC-9® mounting media (Master's Chemical Co., Bensenville, IL), and examined under the light microscope. Most oligochaetes were sent to Mr. M.R. Milligan (Director, Center for Systematics and Taxonomy, Sarasota, FL) for identification confirmation, but some were identified with the aid of keys and reference slides.

Collection and Analysis of Water Data

Water samples were collected from positive ($n$ = 19) and negative sites ($n$ = 19) on a seasonal basis over a 2-yr period (1995 -1997). Samples were collected during 2 wet seasons (March - October) and repeated in 2 dry seasons (November - February) to account for seasonal variation in rainfall input (Chen and Gerber 1990). Site locations and descriptions are presented in Appendix B. All on-site water measurements were made at mid-column (0.25 - 0.50 M), including dissolved oxygen (mg/L), temperature (C), conductivity ($\mu$S/cm), and pH. Dissolved oxygen and temperature were recorded using a YSI® Model 55 DO meter (Yellow Springs Instruments Inc., Yellow Springs, OH), conductivity was measured using a YSI® Model 33 conductivity meter, and pH was recorded with an Oaktron® pHTestr3™

(Forestry Supply Inc., Jackson, MS). Water samples were collected from mid-column in acid-cleaned Nalgene bottles. One additional sample was collected from each site and filtered through a 0.40-$\mu$m milipore membrane filter. Samples were sealed, preserved on ice, and submitted to an E.P.A. certified laboratory for analysis (Dept. of Soil and Water Sciences, Univ. of Fla., Gainesville). Some samples from southern Florida were submitted to Harbor Branch Environmental Laboratory, Fort Pierce, Fla. Samples were analyzed for inorganic carbon (mg/L), total organic carbon (mg/L), total nitrogen (mg/L), total phosphorus (mg/L), and chlorophyll-a (mg/m$^3$) according to standard methods (USEPA 1979, APHA 1985).

Collection and Analysis of Sediment Data

Sediment samples were collected from positive ($n$ = 19) and negative sites ($n$ = 19) on a seasonal basis over a 2-yr period (1995 -1997). Samples were collected during 2 wet seasons (March - October) and repeated in 2 dry seasons (November - February) to account for seasonal variation in rainfall input (Chen and Gerber 1990). Site descriptions are presented in Appendix B. When possible, sediment samples were collected from the littoral zone (within 5 M of

shore), where most wading birds were observed foraging. Sediment samples were collected using a 8.9 (I.D.) x 12.7cm PVC corer. Samples were placed in plastic bags, preserved on ice, and submitted to an E.P.A. certified laboratory for analysis (Dept. of Soil and Water Sciences, Univ. of Fla., Gainesville). Some samples from southern Florida were submitted to Harbor Branch Environmental Laboratory, Fort Pierce, Fla. Samples were analyzed for soil oxygen demand (mg/kg/hr), total phosphorus (mg/kg), total nitrogen (g/kg), and total carbon (g/kg). Additional sediment samples were collected from positive ($n$ = 24) and negative sites ($n$ = 24) for determination of sediment grain size. Because grain size was not expected to change significantly with time (Brown et al. 1990), 1 sample was collected from each site. Samples were dried and filtered through a series of metal sieves (1.0, 0.5, 0.25, 0.106, and 0.045 mm). Amount of sample retained by each sieve was recorded and presented as a percent of total sample and percent composition following USDA (1992).

Collection and Analysis of Vegetation Data

The aquatic macrophyte vegetation types and estimated percent area coverage (PAC) were recorded at positive ($n$ =

30) and negative sites ($n$ = 30) throughout peninsular Florida (Appendix B) following Krebs (1989) and Tiner (1993). Observations were taken during wet and dry seasons at each site. Two 5 m transects were extended at 90° from the shoreline into the water and placed at the lateral boundaries of each specimen collection site. Vegetation was recorded between the transects as percent composition (PC) of emergent, floating, grasses, or submerged. Total PAC was determined as the percent total surface area covered by macrophytes.

Collection and Culture of Eggs of *E. ignotus*

Dead great blue herons (*Ardea herodias*), great egrets (*Ardea albus*), and snowy egrets (*Egretta thula*) were obtained from wildlife rehabilitation facilities in Gainesville (Alachua County), St. Petersburg (Pinellas County), and Key Largo (Monroe County). Some dead birds were obtained from the Wildlife Clinic, College of Veterinary Medicine, Univ. of Fla., Gainesville. At necropsy, gravid female *E. ignotus* were removed from infected birds and placed in petri dishes containing distilled water. Under a dissecting microscope, parasite eggs were removed from the posterior 1/3 of the uteri of worms. The eggs were washed in saline and stored in test

tubes containing distilled water. Eggs were incubated at 28, 22, and 19.5 C and examined daily under a light microscope for development. Some eggs were fixed in hot glycerine alcohol, cleared in glycerine, and examined microscopically. Once eggs contained first-stage larvae, they were used for experimental transmission or stored at 5C for future work.

Measures (1988*c*) reported that fungal growth was detrimental to the survival and development of *E. tubifex* eggs. Initially, I used a fungicide (0.1% formalin) in some cultures, but determined that replacing the culture water on a weekly basis was effective in reducing fungal growth and therefore discontinued the use of fungicide. In addition, Measures (1988*c*) observed that eggs of *E. tubifex* stuck to various surfaces, including pipettes. She treated pipettes with an organosaline surfactant to reduce the problem of transferring eggs. I observed the same adhering characteristics in eggs of *E. ignotus*, but was able to transfer eggs using disposable plastic pipettes.

Collection, Maintenance and Experimental Infection of Oligochaetes

Oligochaetes were collected from the Univ. of Fla. sewage treatment plant outflow area (N 29° 38.451', W 82° 20.978') using a core sampler. This site was chosen because oligochaetes were abundant and infected fishes had been reported from the site prior to the study (Spalding et al. 1993). Bottom sediment was placed in a bucket and transported to the lab. Oligochaetes were counted, identified, and placed in covered culture dishes containing sterile sand, distilled water, and finely chopped spinach. Water was maintained at a depth of 2 cm and replenished as needed. Cultures were incubated at 19.5, 22, and 28 C.

Approximately 500 eggs of *Eustrongylides* containing larvae were added to each experimental oligochaete culture using a plastic pipet. Control cultures did not receive eggs and were maintained for each treatment. Oligochaetes were exposed to eggs for 48-hrs then removed from culture and cleaned of sand, eggs, and organic material. The oligochaetes were returned to incubators in cleaned culture dishes with sterile sand, distilled water, and small amounts of spinach. At 7-day intervals, the oligochaetes were

removed from culture, anesthetized in carbonated water, and examined under a dissecting microscope for parasite development. Some infected oligochaetes were dissected and larvae of *Eustrongylides* were removed at various stages of development. Larvae were fixed in glycerine alcohol, cleared in lactophenol, and examined microscopically. Many infected oligochaetes were retained in culture and fed to experimental fishes for transmission studies. Voucher specimens of *Eustrongylides* larvae were deposited in the U.S. National Parasite Collection, Beltsville, Maryland.

Collection, Maintenance and Experimental Infection of Fishes

Experimental fishes were collected from two sites where *E. ignotus* was never reported. These sites were Watermelon Pond (N29° 33', W82° 25.') and Lake Wauberg (N29° 36', W82° 19') in Alachua County. Control fish ($n$ = 500) were collected and examined from each site also. Fish were captured using seine nets and transported to the lab in aerated coolers. Fish were maintained in aerated 10-gallon aquaria at room temperature (20 - 23 C) and in heated aquaria (26 C). Most aquaria contained sterile sand and tap water only. Some aquaria were maintained with various combinations of aquatic macrophytes, sediment (screened to

remove aquatic invertebrates), and water from the UF sewage treatment plant outflow. Fish were fed commercial food flakes daily. Mosquitofish (*Gambusia holbrooki*), warmouth (*Lepomis gulosus*), blue gill (*L. macrochirus*), largemouth bass (*Micropterus salminoides*), and black crappie (*Pomoxis nigromaculatus*) were used to test for parasite transmission by feeding infected oligochaetes. Individual infected oligochaetes were dropped into aquaria, where they were consumed by fish. After each fish ingested an oligochaete, the fish was isolated using a screen aquarium divider to insure that each animal received only 1 treatment. Fish were killed and examined for parasite larvae at 7-day intervals starting 7-days PI.

Mosquitofish were fed developed parasite eggs also. Most fish were fed eggs mixed with flake fish food and water. This mixture was examined under the light microscope and eggs were observed to adhere to the food. Fish were presumed to consume eggs when they fed on flakes. Developed eggs ($n \approx 10$ per treatment) were injected into the oral cavities of some fish using a feeding tube attached to a 1cc syringe.

To test for biomagnification of the parasite and transmission to paratenic hosts, predatory fish (warmouth, blue gill, largemouth bass, and black crappie) were fed infected mosquitofish. Fresh-killed mosquitofish were dissected to confirm the presence of 1 fourth-stage *E. ignotus* larva. The larva was returned to the coelomic cavity of the mosquitofish which was dropped into an aquarium containing predatory fish. Predators readily consumed both mosquitofish and larva. Some fish were given multiple treatments. Fish were killed and examined for parasites at 30-days PI.

Fish Behavior Study

A 55-gallon aerated aquarium with removable plexiglass divider and remote observation windows was constructed (Fig. 3). The tank was filled with tap water and maintained at 26 C. Aquatic macrophytes (*Sagittaria* spp. and *Hydrilla verticillata*) were placed in the tank to provide refuge for fish. Mosquitofish were collected from an urban retention pond in Alachua County (N 29° 39.4', W 82° 24.5') that was enzootic for *Eustrongylides*. Live fish were examined by transillumination to confirm infection with advanced-stage *Eustrongylides* larvae. One infected and 1 non-infected fish

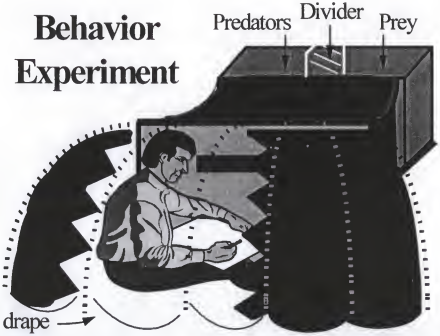


Figure 3. Behavior experiment apparatus. A removable plexiglass divider separated the 55-gallon aquarium. Predatory fish were placed on 1 side of the divider, while 1 mosquitofish (*Gambusia holbrooki*), infected with a larva of *Eustrongylides*, and 1 non-infected mosquitofish were placed on the opposite side. The divider was removed and the observer recorded which prey was selected first, the number of capture attempts, and the time until capture. In addition, predators were observed for 4-days post-ingestion for aberrant behavior.

(of the same gender and approximate size) were introduced to one side of the tank and allowed to acclimate for 24-hrs. Predatory fish (bluegill, largemouth bass, and warmouth) were collected from Watermelon Pond and placed in aerated holding tanks. Two predators (of the same species) were introduced to the opposite side of the test aquarium from mosquitofish and allowed to acclimate for 24-hrs. The divider was then removed and an observer recorded which mosquitofish was selected first, the number of capture attempts, and the time required for capture. Predators were observed for behavioral alterations for 4-days post-ingestion (PI) of infected mosquitofish, then killed and necropsied. Over a 4-month period, 38 trials were performed.

Statistical Analysis

The Chi-square test was used to test for regional differences in the percent of positive sites, the prevalence, and seasonality of infected fish. Alpha level was $P = 0.05$ for all Chi-square tests. Fisher's exact test (Siegel 1956) was used to compare prevalences of infected fish between fish species.

Analysis of variance (ANOVA), square root transformed, was used to test for differences between positive and negative sites (independent variable) with respect to dependent variables: water chemical parameters, sediment chemical parameters, sediment grain size, oligochaete densities, fish densities, and vegetation composition. I used Duncan's multiple range test to compare means among groups with the alpha level set at 0.05 for the rejection of the null hypothesis of equal means (SAS, GLM; SAS Inst. Inc. 1988). The 2-sample *t*-test was used to compare mean sediment grain size between positive and negative sites. In behavioral experiments, selection preference, number of capture attempts, and mean length of capture time for infected and non-infected fish were compared separately using 2-sample *t*-tests. I used simple linear regression to compare changes of individual dependent variables (water chemical parameters, sediment chemical parameters, oligochaete densities, fish abundance, and prevalence of infected fish) with time (independent variable) at the Lake Alice sites.

# RESULTS

## Statewide Survey of Fishes

### Distribution of Infected Fishes

Between August 1994 and October 1997 a survey of wading bird foraging sites ($n$ = 176) was conducted throughout peninsular Florida. Fishes were collected from each site and examined for larval *Eustrongylides*. Sites were classified by region, type, and positive or negative for *Eustrongylides* in intermediate hosts (Table 1). Of the study sites examined, positive fishes were collected from 30 (17.1%). Regionally, there was a northern to southern decline in the percent of positive sites. The percent of positive northern sites (38.9%) were significantly higher than central sites (15.2%) or southern sites (10.3%) ($\chi^2$ = 15.8, df =2, $P$ = 0.0004).

All fishes collected from natural (pristine) sites ($n$ = 52) were negative. Although some of these natural sites may have had limited recreational use, such as boating or fishing, human impact was probably minimal.

Table 1. Regional comparisons of study site types in Florida, 1994 - 1997. Sites with intermediate hosts (fishes) infected with larval *Eustrongylides ignotus* are designated positive (+).

| | Site Type[a] | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Natural | | | Agricultural | | | Urban | | | Sewage | | | Total | | |
| Region | No. Examined | No. (+) | % (+) | No. Examined | No. (+) | % (+) | No. Examined | No. (+) | % (+) | No. Examined | No. (+) | % (+) | No. Examined | No. (+) | % (+) |
| North | 7 | 0 | 0 | 5 | 1 | 20.0 | 16 | 8 | 50.0 | 8 | 5 | 62.5 | 36 | 14 | 38.9 |
| Central | 8 | 0 | 0 | 12 | 2 | 16.7 | 5 | 2 | 40.0 | 8 | 1 | 12.5 | 33 | 5 | 15.2 |
| South | 37 | 0 | 0 | 31 | 5 | 16.1 | 34 | 4 | 11.8 | 5 | 2 | 40.0 | 107 | 11 | 10.3 |
| Total | 52 | 0 | 0 | 48 | 8 | 16.7 | 55 | 14 | 25.5 | 21 | 8 | 38.1 | 176 | 30 | 17.1 |

[a]Site Types: Natural = Pristine lakes, ponds, streams, marshes; Agricultural = Aquatic sities with agricultural or dairy runoff; Urban = Human altered sites such as retention ponds, canals, and sites adjacent to boat ramps; Sewage = Aquatic sites with obvious history of human effluent input.

Infected fishes were observed most often at sites with a history of sewage effluent input (38.1%), followed by urban sites (25.5%), and agricultural sites (16.7%) ($\chi^2$ = 12.8, df = 2, $P$ = 0.0017). All of the positive sewage sites were human constructed and had a long history of effluent input (> 10-yrs). Infected fish were collected from old sewage input canals or retention ponds, but were not observed in recently constructed artificial wetlands receiving human effluent. Two positive sewage sites in northern Florida continued to receive tertiary treated sewage effluent throughout the study, while the remainder of treatment facilities were either shut down recently or retrofitted and water was diverted for other uses, such as irrigation. Most urban sites, such as retention ponds, canals, or sites adjacent to boat ramps, were physically altered by excavation and probably received an array of pollution input including runoff from roads and parking lots, along with fertilizer and pesticides from urban landscaping. Many of the agricultural sites were excavated also, but received input from dairy waste or agriculture fertilizer runoff.

Within regions, the highest percent of sites with infected fishes were observed at sewage or urban sites. In northern Florida, the highest percent of positive sites (62.5%) had sewage input (Fisher's exact test, $P = 0.031$), while in central Florida the percent of positive urban sites (40.0%) was highest (Fisher's exact test, $P = 0.009$). The highest percent of positive sites in southern Florida (40.0) had sewage input (Fisher's exact test, $P = 0.028$).

The percent of positive urban sites declined significantly from northern (50.0%), to central (40.0%), to southern (11.8%) Florida (Fisher's exact test, $P = 0.041$). Although parasites were collected from agricultural sites in all regions, there was no significant difference between the percent of positive agricultural sites between regions (Fisher's exact test, $P = 0.642$).

Species of Fishes Collected

A total of 63,451 (40 species) fishes was collected from 176 sites and examined for larval *Eustrongylides* (Table 2). Eleven of 40 (27.5%) species examined were infected. Although total prevalence was low (0.5%), parasite distribution was over-dispersed, with fishes from some sites

Table 2. Fishes collected from Florida and examined for larvae of *Eustrongylides ignotus*, 1994-1997.

| Species | Common Name | Number Examined | Number Positive for *E. ignotus* |
|---|---|---|---|
| *Ameiurus natalis* | Yellow bullhead catfish | 7 | 0 |
| *Ameiurus nebulosus* | Brown bullhead catfish | 7 | 0 |
| *Belonesox belizanus* | Pike top minnow | 4 | 0 |
| *Centrarchus macropterus* | Flier | 8 | 0 |
| *Cichlasoma bimaculatum* | Black acara | 3 | 0 |
| *Cichlasoma octofasciatum* | Jack dempsey | 3 | 0 |
| *Cyprinodon variegatus* | Sheepshead minnow | 46 | 1 |
| *Dorosoma cepadianum* | Gizzard shad | 35 | 0 |
| *Dorosoma pretenense* | Threadfin shad | 26 | 0 |
| *Elassoma evergladei* | Everglades pygmy sunfish | 34 | 0 |
| *Elassoma zonatum* | Banded pygmy sunfish | 5 | 0 |
| *Enneacanthus gloriosus* | Bluespotted sunfish | 28 | 0 |
| *Erimyzon sucetta* | Lake chubsucker | 1 | 0 |
| *Etheostoma fusiforme* | Swamp darter | 25 | 0 |
| *Fundulus chrysotus* | Golden topminnow | 433 | 0 |
| *Fundulus lineolatus* | Lined topminnow | 34 | 0 |
| *Fundulus seminolis* | Seminole killifish | 8 | 0 |
| *Gambusia holbrooki* | Eastern mosquitofish | 50373 | 296 |
| *Heterandria formosa* | Least killifish | 3336 | 10 |
| *Jordanella floridae* | Flagfish | 433 | 0 |
| *Labidesthes sicculus* | Brook silverside | 337 | 0 |
| *Lepisosteus platyrhincus* | Florida gar | 112 | 1 |
| *Lepomis gulosus* | Warmouth | 272 | 6 |
| *Lepomis macrochirus* | Bluegill | 277 | 3 |
| *Lepomis microlophus* | Redear microlophus | 23 | 0 |
| *Lepomis marginatus* | Dollar sunfish | 23 | 0 |
| *Lepomis punctatus* | Spotted sunfish | 30 | 0 |
| *Liposarcus disjunctivus* | Suckermouth catfish | 1 | 0 |
| *Liposarcus multiradiatus* | Armoured catfish | 2 | 0 |
| *Lucania goodei* | Bluefin killifish | 2378 | 2 |
| *Menidia beryllina* | Inland silverside | 23 | 0 |
| *Micropterus salminoides* | Largemouth bass | 194 | 2 |
| *Notropis hypselopterus* | Sailfin shiner | 455 | 0 |
| *Notropis maculatus* | Taillight shiner | 28 | 0 |
| *Oreochromis aureus* | Blue tilapia | 20 | 0 |
| *Poecilia latipinna* | Sailfin molly | 4004 | 4 |
| *Pomoxis nigromaculatus* | Black crappie | 38 | 1 |
| *Tilapia aurea* | Blue tilapia | 2 | 0 |
| *Tilapia mariae* | Spotted tilapia | 38 | 0 |
| *Xiphophorus variatus* | Variable platyfish | 345 | 5 |
| | Total = | 63451 | 331 |

having high prevalences and those from others having no parasites or very low prevalences.

Poeciliidae (*Belonesox*, *Gambusia*, *Heterandria*, *Poecilia*, and *Xiphophorus*) were the most common fishes observed at most study sites, although primary capture technique (seine net) may have allowed some larger fish to escape. Of the 63,451 fishes collected, 58,062 (91.5%) were poeciliids. Likewise, of the infected fishes collected, 315 of 331 (95%) were poeciliids. *Gambusia*, *Heterandria*, and *Poecilia* are native, while *Belonesox* and *Xiphophorus* are introduced species from South America and were probably released by aquaria owners (Loftus and Kushlan 1987). Poeciliids are the most abundant fishes in the littoral zone of Florida waters and are prey for many aquatic species including birds, fish, amphibians, and reptiles (Loftus and Kushlan 1987). Parasite prevalence appeared to increase with increasing trophic level. At positive sites, 11 species of infected fish were collected (Table 3). Large predatory species included *Lepomis gulosus*, *L. machrochirus*,

Table 3. Total number of fishes examined for larvae of *Eustrongylides* from sites with infected fishes collected in Florida, 1994 -1997.

| Category[a] | Family | Species | No. Examined | No. Positive | Prevalence (%) of Positive Fish | Percent of Infected Fish Collected |
|---|---|---|---|---|---|---|
| Predator | Centrarchidae (sunfish) | *Lepomis gulosus* | 82 | 6 | 7.32 | 1.81 |
| | | *Lepomis machrochirus* | 106 | 3 | 2.83 | 0.91 |
| | | *Micropterus salmoides* | 43 | 2 | 4.65 | 0.60 |
| | | *Pomoxis nigromaculatus* | 18 | 1 | 5.56 | 0.30 |
| | Lepisosteidae (gar) | *Lepisosteus platyrhincus* | 42 | 1 | 2.38 | 0.30 |
| | | Total = | 291 | 13 | 4.47 | 3.93 |
| Prey | Cyprinodontidae (killifish) | *Cyprinodon variegatus* | 26 | 1 | 3.85 | 0.30 |
| | | *Lucania goodei* | 1,142 | 2 | 0.18 | 0.60 |
| | Poeciliidae (livebearers) | *Gambusia holbrooki* | 13,057 | 296 | 2.27 | 89.43 |
| | | *Heterandria formosa* | 1,123 | 10 | 0.89 | 3.02 |
| | | *Poecilia latipinna* | 1,684 | 4 | 0.24 | 1.21 |
| | | *Xiphophorus variatus* | 345 | 5 | 1.45 | 1.51 |
| | | Total = | 17,377 | 318 | 1.83 | 96.07 |

[a]Predatory spp. (Centrarchidae and Lepisosteidae) are reported to consume prey spp. (Cyprinodontidae and Poeciliidae) in Florida (Loftus and Kushlan, 1987).

*Micropterous salmoides*, *Pomoxis migromaculatus*, and *Lepisosteus platyrhincus*. Small prey species were *Cyprinodon variagatus*, *Luciania goodei*, *Gambusia holbrooki*, *Heterandria formosa*, *Poecilis latipina*, and *Xiphosphorus variatus*. The prevalence of *Eustrongylides* sp. in prey species (1.8%) was lower than the prevalence in predators (4.5%) ($\chi^2$ = 11.0, df =1, $P$ = 0.0009). One fish (*L. gulosus*) contained 3 larvae, 9 mosquitofish were infected with 2 larvae each, and the remainder of fishes had single infections. All larvae collected from prey species were coiled tightly, encapsulated, and attached to the mesentary in the coelomic cavity. Capsules were round, pink and measured 5 - 12 mm diameter. Although some larvae from predators ($n$ = 5) were coiled and encapsulated, most were free in the coelomic cavity or had migrated into surrounding tissues and were not encapsulated.

Size and Gender of Infected Mosquitofish

Standard lengths and depths of mosquitofish from positive sites were compared (Fig. 4). Infected fish were smaller in length ($t$ = 3.89, $P$ = 0.0001, df = 13,055) and depth ($t$ = 3.32, $P$ = 0.0009, df = 13,055) than non-infected fish. All infected mosquitofish were adults and had reached

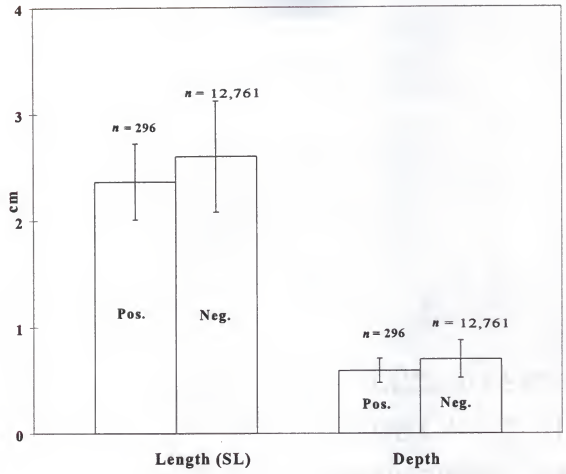


Figure 4. Mean standard length and depth (error bars = SD) of mosguitofish (*Gambusia holbrooki*) collected in Florida, 1994 - 1996. Fish with larvae of *Eustrongylides* are designated positive (Pos.).

reproductive maturity. It could not be determined if infected fish were younger than non-infected fish.

Natural populations of adult mosquitofish are skewed in favor of females (Farr 1989). Although gender ratios appear to be equal at birth, Snelson (1989) suggested that smaller body size and higher mortality of males may result in greater numbers of adult females in most populations. At positive sites, 10,365 of 12,761 (81.2%) were female. Of the infected mosquitofish examined, 234 of 296 (79%) were female. The ratio of infected females to infected males did not differ significantly from the gender ratio observed in the total sample from positive sites ($\chi^2 = 0.89$, df =1, $P = 0.343$) and both genders appeared to be equally susceptible to infection with *Eustrongylides*. Many non-infected female mosquitofish were pregnant and contained well-developed embryos, while infected fish were either not pregnant or contained few embryos.

Seasonal Infections in Fishes

A seasonal comparison of the prevalences of infected mosquitofish at positive sites was made (Fig. 5). The prevalence of infected fish was significantly higher in winter (0.89%) than other seasons ($P < 0.0001$). Prevalence

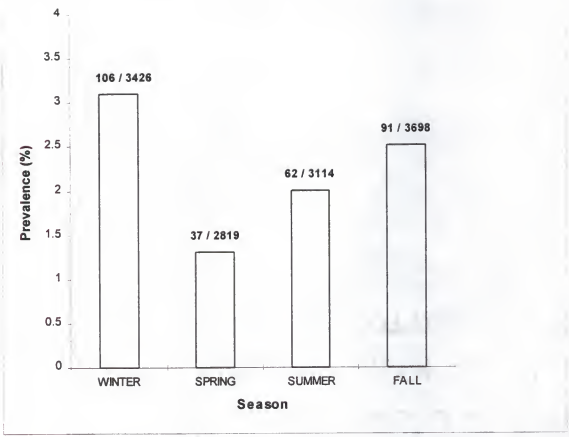


Figure 5. Seasonal comparison of percent *Gambusia holbrooki* infected with larval *Eustrongylides* sp. from study sites in Florida, 1994 - 1997. Seasons: Winter = December 21 - March 20, Spring = March 21 - June 20, Summer = June 21 - September 22, Fall = September 23 - December 20. Numbers at top of bars indicate number infected over sample size for each season.

was lowest in spring (March 21 - June 20) (0.33%), then increased in summer (June 21 - September 22) (0.47%) and fall (September 23 - December 20) (0.67%). Although the number of infected large predatory fishes collected from positive sites was too low for statistical comparison, prevalences were: Winter = 3 of 13 (23%), Spring = 6 of 13 (46%), Summer = 3 of 13 (23%), and Fall = 0.

Fish Densities

Fish densities at positive and negative sites were compared during the dry season (Fig. 6) and wet season (Fig. 7). Densities were significantly higher at positive than negative sites during dry season ($t = 13.8$, $P < 0.0001$, df = 38) and wet season ($t = 14.2$, $P < 0.0001$, df = 38). Regardless of season, fish were easier to catch at positive sites. I found large numbers of fish were often visible near the surface at positive sites and ≥ 100 fish could often be collected with a single sweep of the seine net. Fishes were more dense at all sites during the wet season than during the dry season ($t = 4.24$, $P < 0.0001$, df = 78). Water levels often declined during the dry season and fishes were forced out of vegetation and into deep refugia. Although fish density may have increased in these deeper

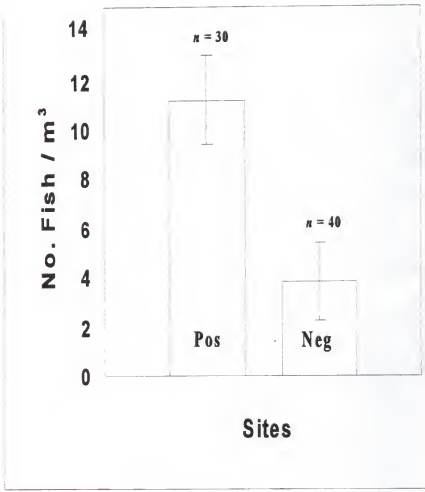


Figure 6. Mean density (error bars = SD) of fishes collected during the dryseason (November - April) at study sites in Florida, 1994 -1997. Sites where fish, infected with larvae of *Eustrongylides*, were collected are designated as positive (Pos.).

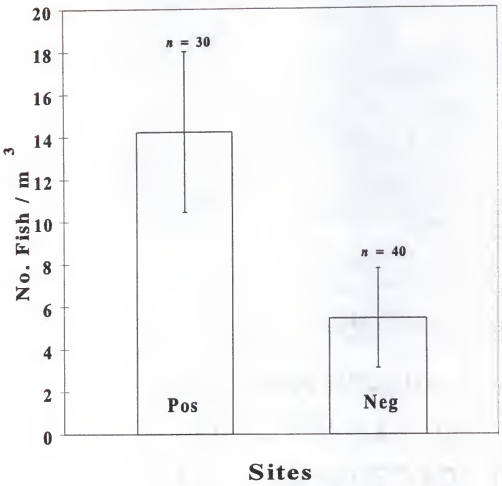


Figure 7. Mean density (error bars = SD) of fishes collected during the wet season (March - October) at study sites in Florida, 1994 -1997. Sites where fish, infected with larvae of *Eustrongylides*, were collected are designated as positive (Pos.).

areas during the dry seasons, the water depth was generally > 3 m and fish were not accessible to foraging wading birds.

Amphibians and Reptiles

A total of 334 amphibians (8 species) and 34 reptiles (9 species) was examined from 11 positive sites and examined also (Table 4). In this study, no infected amphibians or reptiles were collected.

Results of Oligochaete Survey

Oligochaetes ($n$ = 10,508) were collected and identified from positive sites ($n$ = 22) and negative sites ($n$ = 36) (Table 5). Samples were collected from each site during the wet season (April - October) and the dry season (November - March) to account for seasonal variation in total numbers and species composition. Thirty species were identified from positive sites and 28 species were collected at negative sites. In addition, 4,174 of the 10,508 oligochaetes collected (39.7%) were immature tubificids and were classified by the presence or absence of hair setae. Six taxonomic groups (immature Tubificidae without hair setae, *Dero digitata*, *Limnodrilus hoffmeisteri*, *Aulodrilus pigueti*, *immature* Tubificidae with hair setae, and *Pristina*

Table 4. Total number of each species of amphibians and reptiles examined for larvae of *Eustrongylides* collected from Florida, 1994-1997.

| Species | Common Name | Number Examined | Number Positive for E. ignotus |
|---|---|---|---|
| **Amphibians** | | | |
| *Amphium means* | Two-toed amphium | 6 | 0 |
| *Limnaoedus ocularis* | Little grass frog | 14 | 0 |
| *Pseudacris nigrita* | Southern chorus frog | 15 | 0 |
| *Rana catesbeiana* | Bullfrog | 62 | 0 |
| *Rana grylio* | Pig frog | 137 | 0 |
| *Rana sphenocephala* | Leopard frog | 42 | 0 |
| *Notophthalmus viridescens* | Eastern newt | 26 | 0 |
| *Siren lacertina* | Greater siren | 32 | 0 |
| | TOTAL = | 334 | 0 |
| **Reptiles** | | | |
| *Agkistrodon piscivorus* | Cottonmouth snake | 4 | 0 |
| *Chrysemys nelsoni* | Florida red-bellied turtle | 1 | 0 |
| *Kinosternon subrubrum* | Mud turtle | 2 | 0 |
| *Nerodia taxispilota* | Brown water snake | 12 | 0 |
| *Regina alleni* | Striped crayfish snake | 1 | 0 |
| *Seminatrix pygaea* | Swamp snake | 2 | 0 |
| *Thamnophis sauritus* | Eastern ribbon snake | 4 | 0 |
| *Trionyx ferox* | Florida softshell turtle | 5 | 0 |
| *Sternotherus odoratus* | Stinkpot turtle | 3 | 0 |
| | TOTAL = | 34 | 0 |

Table 5. Numbers of aquatic oligochaetes collected from study sites in Florida during wet and dry seasons, 1994 - 1997. Sites are designated positive or negative for the presence of *Eustrongylides* larvae in fishes. Wet season was April - Ocotober, dry season was November - March. Number of sites where each species was collected is indicated (No. Sites). Immature Tubificidae are identified as with or without hair saetae.

| | Positive Sites (22 Sites) | | | | | | Negative Sites (36 Sites) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Wet Season | | Dry Season | | | | Wet Season | | Dry Season | | | |
| Species | No. Collected | No. Sites | No. Collected | No. Sites | Total Specimens Collections | % of Tot. | No. Collected | No. Sites | No. Collected | No. Sites | Total Specimens Collections | % of Tot. |
| Immature Tubificidae w/o hairs | 1615 | 21 | 942 | 18 | 2557 | 41.6 | 824 | 28 | 327 | 19 | 1151 | 26.7 |
| *Dero digitata* | 938 | 16 | 461 | 18 | 1399 | 22.8 | 794 | 21 | 123 | 8 | 917 | 21.3 |
| *Limnodrilus hoffmeisteri* | 526 | 18 | 328 | 17 | 854 | 13.9 | 220 | 9 | 76 | 11 | 296 | 6.9 |
| *Aulodrilus pigueti* | 243 | 14 | 46 | 8 | 289 | 4.7 | 129 | 14 | 17 | 5 | 146 | 3.4 |
| Immature Tubificidae w/ hairs | 186 | 9 | 95 | 6 | 281 | 4.6 | 59 | 6 | 126 | 9 | 185 | 4.3 |
| *Pristina synclites* | 88 | 8 | 101 | 9 | 189 | 3.1 | 352 | 25 | 291 | 18 | 643 | 14.9 |
| *Ilyodrylus templetoni* | 63 | 8 | 61 | 5 | 124 | 2 | 0 | - | 6 | 2 | 6 | < 1 |
| *Haemonais waldvogeli* | 51 | 6 | 48 | 7 | 99 | 1.6 | 182 | 11 | 46 | 7 | 228 | 5.3 |
| *Dero furcata* | 48 | 6 | 16 | 3 | 64 | < 1 | 3 | 1 | 1 | 1 | 4 | < 1 |
| *Dero trifida* | 6 | 2 | 40 | 3 | 46 | < 1 | 86 | 3 | 12 | 4 | 98 | 2.3 |
| *Eclipidrilus* sp. | 38 | 3 | 5 | 2 | 43 | < 1 | 4 | 2 | 0 | - | 4 | < 1 |
| *Aulodrilus limnobius* | 31 | 2 | 5 | 1 | 36 | < 1 | 0 | - | 0 | - | 0 | - |
| *Slavina appendiculata* | 16 | 3 | 19 | 2 | 35 | < 1 | 2 | 1 | 0 | - | 2 | < 1 |
| *Nais pardalis* | 26 | 4 | 12 | 3 | 38 | < 1 | 0 | - | 0 | - | 0 | - |

Table 5. Continued

| Species | Positive Sites (22 Sites) | | | | | | Negative Sites (36 Sites) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Wet Season | | Dry Season | | | | Wet Season | | Dry Season | | | |
| | No. Collected | No. Sites | No. Collected | No. Sites | Total Specimens Collections | % of Tot. | No. Collected | No. Sites | No. Collected | No. Sites | Total Specimens Collections | % of Tot. |
| *Pristina leidyi* | 24 | 5 | 2 | 1 | 26 | < 1 | 135 | 17 | 34 | 9 | 169 | 3.9 |
| *Bratislavia unidentata* | 10 | 2 | 2 | 1 | 12 | < 1 | 4 | 1 | 0 | - | 4 | < 1 |
| *Dero lodeni* | 8 | 2 | 3 | 2 | 11 | < 1 | 0 | - | 0 | - | 0 | - |
| *Lumbriculus variegatus* | 8 | 3 | 0 | - | 8 | < 1 | 58 | 5 | 4 | 2 | 62 | 1.4 |
| *Pristina aequiseta* | 7 | 2 | 1 | 1 | 8 | < 1 | 9 | 2 | 2 | 1 | 11 | < 1 |
| *Branchiura sowerbyi* | 6 | 1 | 0 | - | 6 | < 1 | 0 | - | 0 | - | 0 | - |
| Dero *vega* | 5 | 1 | 0 | - | 5 | < 1 | 0 | - | 0 | - | 0 | - |
| *Nais communis* | 0 | - | 3 | 1 | 3 | < 1 | 0 | - | 0 | - | 0 | - |
| *Pristina proboscidea* | 3 | 1 | 0 | - | 3 | < 1 | 1 | 1 | 1 | 1 | 2 | < 1 |
| *Spirosperma ferox* | 2 | 1 | 0 | - | 2 | < 1 | 1 | 1 | 0 | - | 1 | < 1 |
| *Bothrioneurum vedjdovskyanum* | 1 | 1 | 0 | - | 1 | < 1 | 0 | - | 0 | - | 0 | - |
| *Eclipidrilus palustrus* | 1 | 1 | 0 | - | 1 | < 1 | 1 | 1 | 0 | - | 1 | < 1 |
| *Limnodrilus udekmianus* | 1 | 1 | 0 | - | 1 | < 1 | 0 | - | 0 | - | 0 | - |
| *Naididae* sp. | 0 | - | 1 | 1 | 1 | < 1 | 0 | - | 0 | - | 0 | - |

Table 5. Continued

| Species | Positive Sites (22 Sites) | | | | | | Negative Sites (36 Sites) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Wet Season | | Dry Season | | | | Wet Season | | Dry Season | | | |
| | No. Collected | No. Sites | No. Collected | No. Sites | Total Specimens Collections | % of Tot. | No. Collected | No. Sites | No. Collected | No. Sites | Total Specimens Collections | % of Tot. |
| *Nais variabilis* | 1 | 1 | 0 | - | 1 | < 1 | 156 | 11 | 4 | 2 | 160 | 3.7 |
| *Pristina bidentata* | 1 | 1 | 0 | - | 1 | < 1 | 0 | - | 0 | - | 0 | - |
| *Psammoryctides convolutus* | 1 | 1 | 0 | - | 1 | < 1 | 0 | - | 0 | - | 0 | - |
| *Varichaetadrilus agustipenis* | 1 | 1 | 0 | - | 1 | < 1 | 0 | - | 0 | - | 0 | - |
| *Nais magnaseta* | 0 | - | 0 | - | 0 | - | 66 | 7 | 0 | - | 66 | 1.5 |
| *Haberspeiosus* sp. | 0 | - | 0 | - | 0 | - | 122 | 13 | 56 | 5 | 178 | 4.1 |
| *Stylaria lacustris* | 0 | - | 0 | - | 0 | - | 8 | 2 | 0 | - | 8 | < 1 |
| *Dero vega* | 0 | - | 0 | - | 0 | - | 6 | 2 | 1 | 1 | 7 | < 1 |
| *Psammoryctides convolutus* | 0 | - | 0 | - | 0 | - | 4 | 1 | 1 | 1 | 5 | < 1 |
| *Trieminetia carderoi* | 0 | - | 0 | - | 0 | - | 4 | 1 | 0 | - | 4 | < 1 |
| *Chaetogaster diastrophus* | 0 | - | 0 | - | 0 | - | 0 | - | 1 | 1 | 1 | < 1 |
| *Nais* pardalis | 0 | - | 0 | - | 0 | - | 0 | - | 1 | 1 | 1 | < 1 |
| *Pristina longiseta* | 0 | - | 0 | - | 0 | - | 1 | 1 | 0 | - | 1 | < 1 |
| *Pristinella* sima | 0 | - | 0 | - | 0 | - | 0 | - | 1 | 1 | 1 | < 1 |
| Total | 3955 | | 2191 | | 6146 | | 3231 | | 1131 | | 4362 | |

*synclites*) made up 91% of specimens collected from positive sites and 78% from negative sites. Because of prevalence, these oligochaetes were considered the most important species identified from positive sites. Immature Tubificidae without hair setae, *D. digitata*, *L. hoffmeisteri*, *A. pigueti*, and immature Tubificidae with hair setae were collected from more positive sites and in greater densities than at negative sites (Table 6). *Pristina synclites* was found at more negative sites and in higher densities than at positive sites. Of the remaining species collected from positive sites, *Illyodrylus templetoni* and *Haemonais waldvogeli* each made up ≤ 2%, while all others made up ≤ 1% of the total number of individuals collected and were considered uncommon.

Core samples ($n$ = 162) were collected from natural, agricultural, sewage, and urban sites throughout peninsular Florida. Oligochaetes were counted and mean densities were compared for positive and negative sites (Fig. 8). Natural sites (which were all negative for *Eustrongylides* in intermediate hosts) had the lowest mean (SE) density of oligochaetes [19.8 (10.3)], while sewage sites [113.7 (51.6)] had the highest ($P$ = 0.001). Densities were higher

Table 6. Common aquatic oligochaetes collected from study sites in Florida, 1994 - 1997. Sites are designated positive or negative for the presence of larvae of *Eustrongylides* in fishes.

| Species[a] | % Sites Collected | | Mean (SE) No. Oligochaetes / core (579cm$^3$) | | |
|---|---|---|---|---|---|
| | Pos ($n$ = 22) | Neg ($n$ = 36) | Pos ($n$ = 22) | Neg ($n$ = 36) | Significance |
| Immature Tubificidae w / o hair setae | 21 / 22 (96%) | 28 / 36 (78%) | 58.1 (9.8)* | 15.9 (3.1) | square-root transformed $t$ = 4.9, $P$ < 0.0001, df = 56 |
| *Dero digitata* | 18 / 22 (82%) | 22 / 36 (61%) | 31.8 (6.5)* | 12.7 (2.0) | square-root transformed $t$ = 3.3, $P$ = 0.0007, df = 56 |
| *Limnodrilus hoffmeisteri* | 19 / 22 (86%) | 12 / 36 (22%) | 19.4 (4.4)* | 4.1 (1.2) | square-root transformed $t$ = 4.4, $P$ < 0.0001, df = 56 |
| *Aulodrilus pigueti* | 16 / 22 (73%) | 15 / 36 (42%) | 6.6 (1.4)* | 2.0 (0.6) | square-root transformed $t$ = 1.4, $P$ = 0.0004, df = 56 |
| Immature Tubificidae w / hair setae | 9 / 22 (41%) | 10 / 36 (28%) | 6.4 (1.9)* | 2.6 (0.6) | square-root transformed $t$ = 2.5, $P$ = 0.0001, df = 56 |
| *Pristina synclites* | 10 / 22 (46%) | 28 / 36 (78%) | 4.3 (1.2) | 8.9 (1.4) | square-root transformed $t$ = 4.9, $P$ < 0.0001, df = 56 |

[a]Density greater ($p \leq 0.05$) at positive sites than negative sites

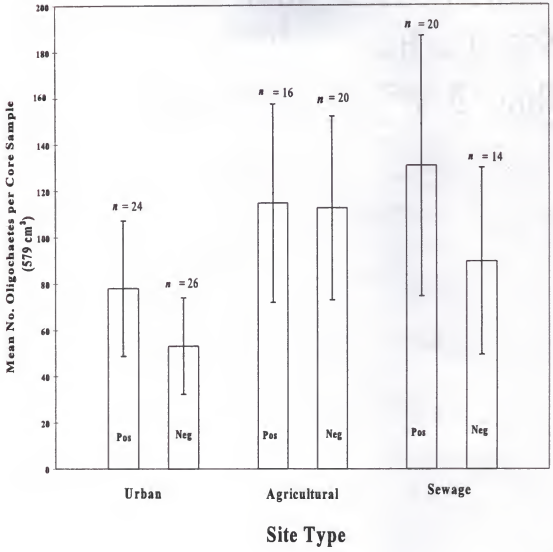


Figure 8. Mean density (error bars = SE) of oligochaetes collected at selected study sites (Urban, Agricultural, and Sewage) in Florida, 1994 - 1997. Sites are positive or negative for larvae of *Eustrongylides* in fishes.

at positive than negative urban sites (square-root transformed $t = 5.8$, $P < 0.0001$, df = 38). Positive sewage sites had higher densities of oligochaetes than negative sewage sites (square-root transformed $t = 2.6$, $P = 0.0175$, df = 19), while there was no significant difference between densities at positive and negative agricultural sites (square-root transformed $t = 0.9$, $P = 0.38$, df = 28).

Physico-chemical Characteristics of Water

Water samples were collected seasonally from 1995 to 1997 from study sites throughout peninsular Florida (Tables 7 - 10). Eleven parameters were analyzed, including dissolved oxygen (DO),pH, conductivity, temperature, inorganic carbon (IC), total organic carbon (TOC), total carbon (TC), total nitrogen (TN), total phosphorus (TP), nitrogen / phosphorus, and chlorophyll-a.

Dissolved oxygen was lower ($P < 0.01$) at positive sites than at negative sites during all seasons. Some positive sites had DO levels of < 1 mg/l. I attempted to test sites in late afternoon (14:00 - 17:00) and on days of full sun, to compensate for diurnal photosynthetic fluctuations in DO, although this appeared to make little difference at most positive sites. Some positive sites, which had large algae

Table 7. Physico-chemical characteristics of water samples collected during the wet season (April - Ocotober), 1995 from study sites in Florida. Sites are designated positive or negative for larvae of *Eustrongylides* in fishes.

| Parameter | Positive sites $\bar{x}$ (SE) | Negative sites $\bar{x}$ (SE) | Significance |
|---|---|---|---|
| | $n = 19$ | $n = 19$ | |
| Dissolved Oxygen (mg/l)[a] | 1.89 (0.39) | 5.14 (0.26) | $t = 6.826$, 36 df, $P < 0.001$ |
| pH | 7.61 (0.14) | 7.40 (0.06) | $t = 1.324$, 36 df, $P = 0.194$ |
| Conductivity ($\mu$mhos/cm) | 361.47 (52.39) | 277.52 (27.95) | $t = 1.141$, 36 df, $P = 0.166$ |
| Temperature (C°) | 27.08 (0.32) | 26.94 (0.41) | $t = 0.272$, 36 df, $P = 0.787$ |
| Inorganic Carbon (mg/l) | 23.27 (3.17) | 23.72 (3.05) | $t = 0.102$, 36 df, $P = 0.918$ |
| Total Organic Carbon (mg/l) | 19.48 (1.93) | 19.81 (1.53) | $t = 0.134$, 36 df, $P = 0.894$ |
| Total Carbon (mg/l) | 42.75 (3.77) | 43.53 (3.26) | $t = 0.156$, 36 df, $P = 0.876$ |
| Total Nitrogen (mg/l)[a] | 2.91 (0.34) | 1.33 (0.14) | $t = 4.261$, 36 df, $P < 0.001$ |
| Total Phosphorus (mg/l)[a] | 0.74 (0.18) | 0.20 (0.06) | $t = 2.739$, 36 df, $P = 0.009$ |
| Nitrogen / Phosphorus[a] | 7.98 (1.44) | 30.08 (6.49) | $t = 3.244$, 36 df, $P = 0.002$ |
| Chlorophyll-a ($mg/m^3$)[a] | 115.89 (15.36) | 41.16 (5.97) | $t = 4.534$, 36 df, $P < 0.001$ |

[a]Significant difference ($P \leq 0.05$) between positive and negative sites.

Table 8. Physico-chemical characteristics of water samples collected during the dry season (November 1995 - March 1996), from study sites in Florida. Sites are designated positive or negative for larvae of *Eustrongylides* in fishes.

| Parameter | Positive sites $\bar{x}$ (SE) | Negative sites $\bar{x}$ (SE) | Significance |
|---|---|---|---|
| | $n = 19$ | $n = 19$ | |
| Dissolved Oxygen (mg/l)[a] | 2.22 (0.51) | 4.900 (0.25) | $t = 4.725$, 36 df, $P < 0.001$ |
| pH | 7.75 (0.18) | 7.34 (0.09) | $t = 1.982$, 36 df, $P = 0.055$ |
| Conductivity ($\mu$mhos/cm) | 324.32 (47.22) | 310.37 (36.59) | $t = 0.233$, 36 df, $P = 0.817$ |
| Temperature (C°) | 21.83 (0.53) | 22.51 (0.39) | $t = 1.018$, 36 df, $P = 0.315$ |
| Inorganic Carbon (mg/l) | 23.39 (3.21) | 26.37 (3.72) | $t = 0.606$, 36 df, $P = 0.548$ |
| Total Organic Carbon (mg/l) | 18.86 (1.92) | 21.02 (1.52) | $t = 0.886$, 36 df, $P = 0.382$ |
| Total Carbon (mg/l) | 42.24 (3.41) | 47.39 (4.14) | $t = 0.960$, 36 df, $P = 0.343$ |
| Total Nitrogen (mg/l)[a] | 2.99 (0.61) | 1.61 (0.24) | $t = 2.105$, 36 df, $P = 0.042$ |
| Total Phosphorus (mg/l)[a] | 0.68 (0.16) | 0.28 (0.11) | $t = 2.148$, 36 df, $P = 0.039$ |
| Nitrogen / Phosphorus[a] | 10.58 (2.17) | 31.19 (6.33) | $t = 3.082$, 36 df, $P = 0.004$ |
| Chlorophyll-a (mg/m$^3$)[a] | 99.84 (14.48) | 30.84 (6.54) | $t = 4.342$, 36 df, $P < 0.001$ |

[a]Significant difference ($P \leq 0.05$) between positive and negative sites.

Table 9. Physico-chemical characteristics of water samples collected during the wet season (April - Ocotober 1996), from study sites in Florida. Sites are designated positive or negative for larvae of *Eustrongylides* in fishes.

| Parameter | Positive sites $\bar{x}$ (SE) | Negative sites $\bar{x}$ (SE) | Significance |
|---|---|---|---|
| | $n = 19$ | $n = 19$ | |
| Dissolved Oxygen (mg/l)[a] | 2.66 (0.48) | 5.57 (0.39) | $t = 4.683$, 36 df, $P < 0.001$ |
| pH | 7.45 (0.09) | 7.34 (0.06) | $t = 1.006$, 36 df, $P = 0.321$ |
| Conductivity ($\mu$mhos/cm) | 347.89 (41.27) | 267.63 (32.09) | $t = 1.535$, 36 df, $P = 0.133$ |
| Temperature (C°) | 27.43 (0.33) | 27.93 (0.41) | $t = 0.272$, 36 df, $P = 0.787$ |
| Inorganic Carbon (mg/l) | 26.14 (2.71) | 18.94 (2.62) | $t = 1.191$, 36 df, $P = 0.064$ |
| Total Organic Carbon (mg/l) | 21.91 (2.46) | 17.84 (1.63) | $t = 1.375$, 36 df, $P = 0.177$ |
| Total Carbon (mg/l)[a] | 48.05 (3.79) | 36.79 (3.33) | $t = 2.231$, 36 df, $P = 0.032$ |
| Total Nitrogen (mg/l)[a] | 2.55 (0.54) | 0.96 (0.10) | $t = 2.911$, 36 df, $P = 0.006$ |
| Total Phosphorus (mg/l)[a] | 0.56 (0.11) | 0.16 (0.05) | $t = 3.271$, 36 df, $P = 0.002$ |
| Nitrogen / Phosphorus[a] | 6.11 (1.05) | 29.65 (7.15) | $t = 3.255$, 36 df, $P = 0.003$ |
| Chlorophyll-a ($mg/m^3$)[a] | 100.57 (14.58) | 45.16 (7.73) | $t = 3.358$, 36 df, $P = 0.002$ |

[a]Significant difference ($P \leq 0.05$) between positive and negative sites.

Table 10. Physico-chemical characteristics of water samples collected during the dry season (November 1996 - March 1997) from study sites in Florida. Sites are designated positive or negative for larvae of *Eustrongylides* in fishes.

| Parameter | Positive sites $\bar{x}$ (SE) | Negative sites $\bar{x}$ (SE) | Significance |
|---|---|---|---|
| | $n = 19$ | $n = 19$ | |
| Dissolved Oxygen (mg/l)[a] | 2.25 (0.39) | 5.10 (0.39) | $t = 5.158$, 36 df, $P < 0.001$ |
| pH | 7.66 (0.17) | 7.38 (0.05) | $t = 1.587$, 36 df, $P = 0.121$ |
| Conductivity ($\mu$mhos/cm) | 374.95 (50.38) | 288.21 (33.83) | $t = 1.429$, 36 df, $P = 0.162$ |
| Temperature (C°) | 23.74 (0.49) | 23.47 (3.30) | $t = 0.458$, 36 df, $P = 0.649$ |
| Inorganic Carbon (mg/l) | 26.12 (2.95) | 24.02 (3.77) | $t = 0.438$, 36 df, $P = 0.663$ |
| Total Organic Carbon (mg/l) | 23.66 (3.73) | 19.63 (1.66) | $t = 0.985$, 36 df, $P = 0.331$ |
| Total Carbon (mg/l) | 49.78 (3.24) | 43.66 (3.89) | $t = 0.117$, 36 df, $P = 0.234$ |
| Total Nitrogen (mg/l)[a] | 3.032 (0.62) | 1.31 (0.17) | $t = 2.680$, 36 df, $P = 0.011$ |
| Total Phosphorus (mg/l)[a] | 0.57 (0.12) | 0.200 (0.06) | $t = 2.809$, 36 df, $P = 0.007$ |
| Nitrogen / Phosphorus[a] | 8.38 (1.64) | 27.35 (6.12) | $t = 2.994$, 36 df, $P = 0.005$ |
| Chlorophyll-a (mg/m$^3$)[a] | 76.11 (11.46) | 27.32 (4.42) | $t = 3.973$, 36 df, $P < 0.001$ |

[a]Significant difference ($P \leq 0.05$) between positive and negative sites.

blooms, were characterized by elevated DO (> 12 mg/l) on days of full sun, but these declined to anoxic concentrations when measured in the early morning or on cloudy days. At many of the hypoxic sites, large fish were colleced rarely and although mosquitofish were often abundant, they were observed frequently at the surface "gulping" air. Hypoxic sites were often human-made canals or retention ponds with restricted waterflow (eg. slow current in canals) and were often protected from the wind by high earthen banks or vegetation.

Although pH did not differ between positive and negative sites at $P < 0.050$, during the dry season of 1995, pH at positive sites was nearly significantly higher at $P = 0.055$. Conductivity, temperature, IC, and TOC did not differ significantly between positive and negative sites during any collection period.

Total carbon was higher at positive sites ($P = 0.032$) in the wet season of 1996, although no difference was observed during other seasons. It was unclear why TC concentrations were elevated that season, but 1996 was a drought year in Florida and most study sites received decreased amounts of rainfall.

Total nitrogen values were higher at positive sites during all seasons ($P < 0.05$). Many of the positive sites were in human-altered watersheds, such as sewage outflows and retention ponds, where they probably received exogenous nitrogen from runoff. Most phosphorus enters aquatic systems from atmospheric precipitation and surface runoff (Gale et al. 1994), and TP was higher at positive than negative sites throughout the study ($P < 0.004$).

Nitrogen / phosphorus ratios were significantly lower at positive sites ($P < 0.001$) throughout the study. Ratios were $\leq 10.6 : 1$ at positive sites and $>27 : 1$ at negative sites. Chlorophyll-a concentrations were $\geq 2$ times higher at positive than negative sites ($P \leq 0.002$). Many positive sites appeared to have active algal blooms throughout the study and water clarity was poor.

Based on increased TN, TP, and chlorophyll-a concentrations, most positive sites were considered eutrophic or hypereutrophic, while many negative sites were oligotrophic or mesotrophic (Wetzel 1983).

Physico-chemical Characteristics of Sediment

Sediment samples were collected seasonally (1995 - 1997) from study sites throughout peninsular Florida (Tables 11 - 14). Sediments were analyzed for total carbon (TC), total nitrogen (TN), and total phosphorus (TP). In addition, soil oxygen demand was measured at 23 positive and 23 negative sites during 1995 - 1996.

Sediment TC and TN did not differ significantly between positive and negative sites throughout the study. Sediment from some positive sites, such as sewage treatment plant outflows and agricultural areas on Lake Apopka were composed of fine particulate floc.

Sediment TP was higher at positive sites during all collection periods ($P \leq 0.027$). Within positive sites and within negative sites, TP did not vary between seasons ($F = 0.044$, $P = 0.987$, $df = 75$; $F = 0.442$, $P = 0.723$, $df = 75$ respectively). Mean soil oxygen demand was 2.6 times higher at positive sites than negative sites during the dry season 1995 -1996 ($P < 0.001$). Because of difficulties associated with rapid submission of specimens to the laboratory (especially from southern Florida), SOD was collected during this season only (Table 12).

Table 11. Results of analysis of sediment collected during the wet season (April - Ocotober), 1995 from study sites in Florida. Sites are designated positive or negative for larvae of *Eustrongylides* in fishes.

| Parameter | Positive sites $\bar{x}$ (SE) | Negative sites $\bar{x}$ (SE) | Significance |
|---|---|---|---|
| | $n = 19$ | $n = 19$ | |
| Total Carbon (g/kg) | 40.13 (7.66) | 39.55 (15.99) | $t = 0.032$, 36 df, $P = 0.974$ |
| Total Nitrogen (g/kg) | 2.91 (0.39) | 3.62 (1.50) | $t = 0.452$, 36 df, $P = 0.654$ |
| Total Phosphorus (mg/kg)[a] | 1680.42 (408.93) | 254.82 (40.53) | $t = 3.469$, 36 df, $P = 0.001$ |

[a] Significant difference ($P \leq 0.05$) between positive and negative sites.

Table 12. Results of analysis of sediment collected during dry season (November 1995 - March 1996) from study sites in Florida. Sites are designated positive or negative for larvae of *Eustrongylides* in fishes.

| Parameter | Positive Sites $\bar{x}$ (SE) | | Negative Sites $\bar{x}$ (SE) | | Significance |
|---|---|---|---|---|---|
| | *n* | | *n* | | |
| Total Carbon (g/kg) | 19 | 30.83 (6.59) | 19 | 27.50 (10.42) | $t = 0.270$, 36 df, $P = 0.789$ |
| Total Nitrogen (g/kg) | 19 | 2.12 (0.33) | 19 | 3.04 (1.20) | $t = 0.739$, 36 df, $P = 0.464$ |
| Total Phosphorus (mg/kg)[a] | 19 | 1487.90 (382.78) | 19 | 207.19 (33.15) | $t = 3.333$, 36 df, $P = 0.002$ |
| Soil Oxygen Demand (mg/kg/hr)[a] | 23 | 20.69 (1.33) | 23 | 7.89 (0.64) | $t = 8.673$, 44 df, $P < 0.001$ |

[a] Significant difference ($P \leq 0.05$) between positive and negative sites.

Table 13. Results of analysis of sediment collected during the wet season (April - Ocotober), 1996 from study sites in Florida. Sites are designated positive or negative for larvae of *Eustrongylides* in fishes.

| Parameter | Positive Sites $\bar{x}$ (SE) | Negative Sites $\bar{x}$ (SE) | Significance |
|---|---|---|---|
| | $n = 19$ | $n = 19$ | |
| Total Carbon (g/kg) | 36.15 (7.57) | 32.45 (13.21) | $t = 0.242$, 36 df, $P = 0.809$ |
| Total Nitrogen (g/kg) | 3.23 (0.53) | 3.32 (1.37) | $t = 0.059$, 36 df, $P = 0.953$ |
| Total Phosphorus (mg/kg)[a] | 1578.12 (349.22) | 257.189 (36.27) | $t = 3.672$, 36 df, $P = 0.001$ |

[a] Significant difference ($P \leq 0.05$) between positive and negative sites.



Table 14 . Results of analysis of sediment collected during the dry season (November 1996 - March 1997), from study sites in Florida. Sites are designated positive or negative for larvae of *Eustrongylides* in fishes.

| Parameter | Positive Sites $\bar{x}$ (SE) | Negative Sites $\bar{x}$ (SE) | Significance |
|---|---|---|---|
| | $n = 19$ | $n = 19$ | |
| Total Carbon (g/kg) | 35.95 (7.52) | 39.62 (17.42) | $t = 0.193$, 36 df, $P = 0.848$ |
| Total Nitrogen (g/kg) | 2.21 (0.37) | 3.40 (1.44) | $t = 2.210$, 36 df, $P = 0.428$ |
| Total Phosphorus (mg/kg)[a] | 1636.82 (425.49) | 263.72 (45.19) | $t = 3.209$, 36 df, $P = 0.027$ |

[a] Significant difference ($P \leq 0.05$) between positive and negative sites.

## Sediment Grain Size

Sediments from selected positive ($n$ = 24) and negative ($n$ = 24) sites were collected and compared for grain (particle) size (Fig. 9) and percent composition (Fig. 10). Mean (SE) grain size at positive sites [0.081 (0.002) mm]was larger than at negative sites [0.058 (0.001) mm] (t-test for unequal variances: $t = 7.23$, $P < 0.0001$, df = 35). Although mean particle size at positive sites was larger, there was more variability in particle sizes at positive sites also. Sediment from positive sites contained higher percent of coarse (0.6 - 1.0 mm) and very coarse (> 1.0 mm) particles than negative sites (which accounted for larger mean grain size), however, positive sites contained higher percent of very fine particles (≤ 0.1 mm) also ($\chi 2 = 14.5$, $P = 0.006$, df = 4). Most particles at negative sites were either fine (0.1 - 0.25 mm) or medium (0.26 - 0.5 mm), with low percents of very fine and very coarse particles. Percent composition (sand, silt, and clay) were calculated for positive and negative sites (Fig. 9). Sand made up the highest mean percent of particles at both positive (87.9%) and negative (95.1%) sites. At positive sites, clay

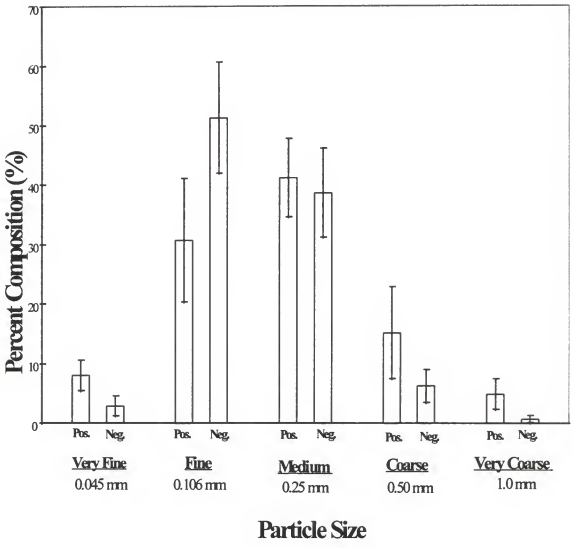


Figure 9. Mean percent sediment composition particle size at study sites. Sites are designated Pos (+) ($n$ = 24) or Neg (-) ($n$ = 24) for larvae of *Eustrongylides* in fish. Very Fine: ≤ 0.1 mm, Fine: 0.1 - 0.25 mm, Medium: 0.26 - 0.5 mm, Coarse: 0.6 - 1.0 mm, Very Coarse: > 1.0 mm.

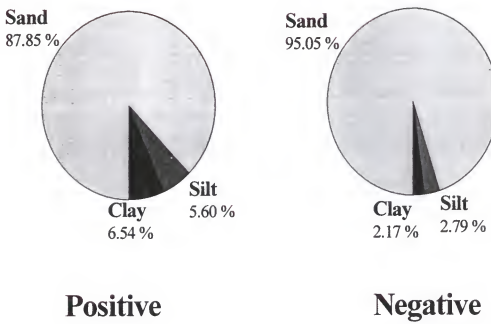


Figure 10. Percent composition of sediment at selected study study sites. Sites are designated designated Pos (+) ($n = 24$) or Neg (-) ($n = 24$) for larvae of *Eustrongylides* in fish.

particles were second most abundant (6.5%), with silt making up the remainder (5.6%). I observed that all watersheds at positive sites were excavated, exposing underlying clay and probably resulting in increased siltation. At negative sites, mean amounts of silt and clay were 2.8% and 2.2% respectively.

Results of Vegetation Survey

Mean aquatic macrophyte percent area coverage (PAC) was calculated at positive and negative sites during wet and dry seasons 1996 - 1997. During wet season (March - October), PAC was higher at negative (49.7%) than positive sites (18.7%) ($\chi^2 = 21.3$, $P < 0.0001$, df = 1). Although PAC at both site types declined during the dry season, PAC remained higher at negative (28.6%) than positive sites (15.6%) ($\chi^2 = 4.9$, $P = 0.0264$, df = 1). I observed dense floating vegetation frequently at many negative sites and this probably resulted in higher mean PAC at these sites.

Vegetation was classified by percent composition at positive and negative sites during wet (Fig. 11) and dry (Fig. 12) seasons also. During the wet season, percent emergent vegetation and grasses (including rushes and sedges) was higher at positive sites, while percent floating

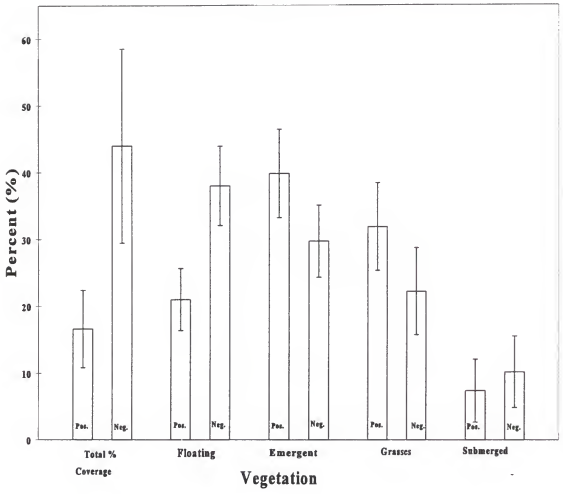


Figure 11. Mean percent vegetation composition during wet season (April - October, 1996) at study sites in Florida. Sites are designated Pos. ($n$ = 24) or Neg. ($n$ = 24) for larvae of *Eustrongylides* in fish. Error bars = SE.

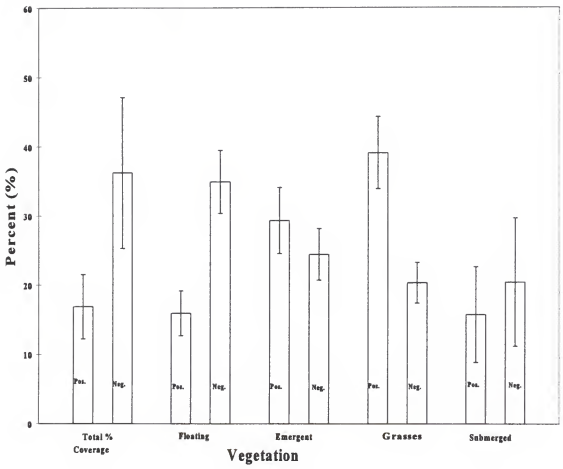


Figure 12. Mean percent vegetation composition during dry season (November, 1996 - March, 1997) at study sites in Florida. Sites are designated Pos ($n = 24$) or Neg ($n = 24$) for larvae of *Eustrongylides* in fish. Error bars = SE.

and sedges. At positive sites, dominant floating vegetation was water-hyacinth (*Eichhornia crassipes*) and common duckweed (*Limna minor*). Floating plants at negative sites included water-lettuce (*Pistia stratiotes*), water-hyacinth, duckweed (*Limna* spp.), bog-mat (*Wolffiella floridana*), and frog's-bit (*Stratiotes aloides*).

The most common emergent plants at all study sites were alligator weed (*Alternanthera philoxeroides*), arrowheads (*Sagittaria* spp.), blue flag (*Iris virginica*), cattail (*Typha* spp.), fire flag (*Thalia geniculata*), pickerelweed (*Pontederia lanceolata*), and spatterdock (*Nuphar luteum*). Infected fish were collected rarely near monospecific stands of cattail, although some positive sites did have a sparse mixture of cattails and other emergents.

Grasses, rushes, and sedges were more common at positive sites than negative sites. Dominant species were cupscalegrass (*Sacciolepis striata*), barnyardgrasses (*Echinochloa* spp.), cordgrasses (*Spartina* spp.), beak-rushes (*Rhynchospora* spp.), bog rushes (*Juncus* spp.), bulrushes (*Scirpus* spp.), maidencane (*Panicum hemitomon*) and white-toped sedge (*Dichromena colorata*). Many of the positive sites ($n$ = 19) were human-disturbed canals or retention

ponds where these plant communities appeared to be in early successional stages after disturbance. At positive sites, grasses and rushes appeared to provide minimal concealment for fish and other aquatic fauna, which were observed near the surface frequently. Water depths at positive sites were often < 1 m and wading birds foraged in grasses and emergent vegetation near the shore.

I collected infected fish infrequently in areas with large amounts of submerged vegetation. Although fish were present, they were often difficult to catch. Submerged plants were located frequently in deep water (> 2 m) where emergent plants were thinly distributed. Dominant submerged species at study sites were hydrilla (*Hydrilla verticillata*), coontail (*Ceratophyllum demersum*), and watermilfoil (*Myriophyllum* spp.).

## Results of Lake Alice Study

### Fish Survey

A total of 6,307 fishes was collected from 4 study sites in the Lake Alice watershed, 1994 - 1997 (Table 15). Nine species of fishes were collected. Poeciliids

Table 15. Fish examined for larvae of *Eustrongylides* from Lake Alice study sites, Florida, 1995 - 1997.

| Species | Site A[a] No. Examined | Site A[a] No. Positive | Site B[b] No. Examined | Site B[b] No. Positive | Site C[c] No. Examined | Site C[c] No. Positive | Site D[d] No. Examined | Site D[d] No. Positive | Total No. Examined | Total No. Positive |
|---|---|---|---|---|---|---|---|---|---|---|
| *Elssoma zonatum* | 0 | - | 0 | - | 1 | 0 | 8 | 0 | 9 | 0 |
| *Fundulus chrysotus* | 8 | 0 | 0 | - | 14 | 0 | 12 | 0 | 34 | 0 |
| *Gambusia holbrooki* | 926 | 3 | 2388 | 38 | 1194 | 16 | 923 | 0 | 5431 | 57 |
| *Heterandria formosa* | 39 | 0 | 102 | 2 | 78 | 1 | 61 | 0 | 280 | 3 |
| *Lepomis gulosus* | 6 | 0 | 9 | 1 | 3 | 0 | 7 | 0 | 25 | 1 |
| *Lepomis macrochirus* | 0 | - | 6 | 0 | 2 | 0 | 4 | 0 | 12 | 0 |
| *Micropterus salminoides* | 0 | - | 0 | - | 0 | - | 8 | 0 | 8 | 0 |
| *Poecilia latipina* | 74 | 0 | 209 | 1 | 156 | 1 | 67 | 0 | 506 | 2 |
| *Talapia aurea* | 1 | 0 | 0 | - | 0 | - | 1 | 0 | 2 | 0 |
| Total | 1054 | 3 | 2714 | 42 | 1448 | 19 | 1091 | 0 | 6307 | 63 |

[a] Site A - 0.5 km upstream from treatment plant - fish positive for larvae of *Eustrongylides*.
[b] Site B - Sewage treatment plant outflow - fish positive for larvae of *Eustrongylides*.
[c] Site C - 0.5 km downstream from sewage treatment plant - fish positive for larvae of *Eustrongylides*.
[d] Site D - Outflow from Lake Alice - fish negative for larvae of *Eustrongylides*.

(mosquitofish, least killifish, and sailfin mollies) made up the largest percentage of fishes collected (98%) and Infected fishes were mosquitofish: 57/5431 (1.1%), least killifish: 3/280 (1.1%), sailfin mollies: 2/508 (0.4%), and warmouth: 1/25 (4%). Of all infected fish collected, 42/64 (65.6%) were collected near the sewage outflow (Site B), 19/64 29.7%) downstream from the outflow (Site C), and 3/63 (4.7%) upstream from the outflow (Site A). No infected fish were collected from the western end of the lake (Site D). The number of infected fish declined after construction of the new sewage treatment plant, total prevalences were A: 3 of 1054 (0.29%), B: 42 of 2714 (2.9%), C: 19 of 1448 (1.3%), and D: 0 of 1091.

Beginning in late 1994 and continuing through 1996, the prevalence of infected fish declined in the watershed (Fig. 13). All infected fish had single infections of *Eustrongylides* larvae. At study site A, 1 of 79 (1.3%) fish was infected on March 6, 1995 and 2 of 111 (1.8%) were infected on June 28, 1995. Following the June 28, 1995 collection, no infected fish were collected at site A. At study site B, 13 of 40 (32.5%) fish were infected on

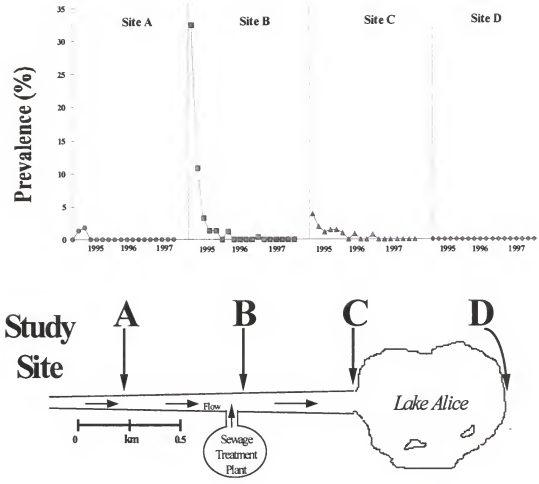


Figure 13. Prevalence (%) of fish infected with larvae of *Eustrongylides* at Lake Alice study sites, Florida, 1994 - 1997. Initial collection was December 23, 1994. Sewage treatment plant was replaced in November, 1994 and effluent was diverted for irrigation. Mean (SD) No. fish / collection were: Site A = 59 (11.3), Site B = 151 (22.8), Site C = 81 (14.5), Site D = 61 (16.2).

December 23, 1994. This prevalence declined to 17 of 157 (10.8%) on March 6, 1995 and 5 of 150 (3.3%) on June 28, 1995. Prevalences continued to decline at B through December 5, 1995, when 3 of 244 (1.2%) fish were infected. Although 1 of 234 (0.4%) infected fish was collected on May 4, 1996, no infected fish were observed at subsequent collections. At study site C, prevalences declined from 2 of 52 (3.8%) on December 23, 1994 to 1 of 137 (0.7%) on November 21, 1996. Although infected fish appeared to remain in the system longer at site C than any other site, they were not detected after November, 1996.

Mean (SE) no. fish / $m^3$ at sites were: A: 7.2 (1.3) $n$ = 18, B: 11.8 (2.1) $n$ = 18, C: 11.7 (1.9) $n$ = 18, D: 9.2 (2.2) $n$ = 18. There was no significant difference in densities of fishes between sites ($F = 1.31$, $P = 0.28$, df = 79). Between winter, 1995 and summer, 1996 fish densities appeared to decline at positive sites (Fig. 14). Fish densities appeared to increase at all sites during 1997.

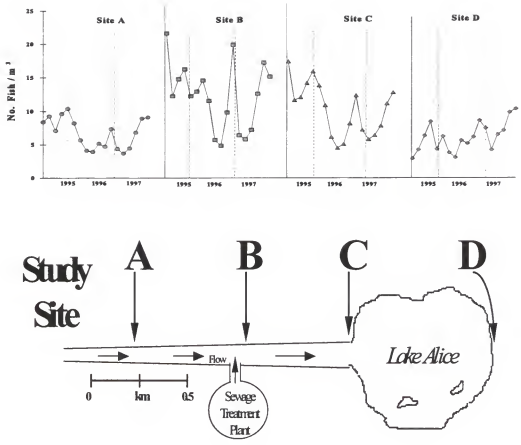


**Figure 14.** Density of fish at Lake Alice study sites, Florida, 1994 - 1997. Initial collection was December 23, 1994. Sewage treatmentplant was retrofit November, 1994 and effluent was diverted for irrigation.

Oligochaete Survey

Oligochaetes ($n$ = 2,093) were collected and identified from the 4 study sites (Table 16). Densities ($\bar{x}$ ± SD) of oligochaetes (no. oligochaetes / 579 $cm^3$) were greater at sites B (43.5 ± 16.9) and C (32.7 ± 9.3) than sites A (17.0 ± 6.1) and D (23.1 ± 6.5) ($P$ = 0.041). Densities did not change significantly throughout the study. A total of 17 taxonomic groups, including 15 species of oligochaetes was identified. Immature Tubificidae without hair setae, *L. hoffmeisteri*, and *D. digitata* were collected from all sites. Immature Tubificidae without hair setae made up 625 of 1677 (37%) of all oligochaetes collected from positive sites (A, B, and C), but only 54 of 416 (13%) of those collected from site D. *Limnodrilus hoffmeisteri* were 286 of 1677 (17%) of oligochaetes collected from positive sites, and 6 of 416 (1.4%) at site D. The most common oligochaete species at site D 184 of 416 (44%) was *D. digitata*. *Dero digitata* made up 340 of 1677 (20%) of oligochaetes collected from positive sites. Immature Tubificidae with hair setae, *Aulodrilus*

Table 16. Oligochaetes collected from Lake Alice study site 1994 - 1997. Samples per site ($n$ = 18). Core samples = 579 $cm^3$.

| Species | Site A[a] | | Site B[b] | | Site C[c] | | Site D[d] | |
|---|---|---|---|---|---|---|---|---|
| | No. Collected | Percent of Total | No. Collected | Percent of Total | No. Collected | Percent of Total | No. Collected | Percent of Total |
| Immature Tubificidae w/o hairs | 103 | 33.7 | 353 | 45.1 | 169 | 28.7 | 54 | 13 |
| *Limnodrilus hoffmeisteri* | 71 | 23.2 | 118 | 15.1 | 97 | 16.5 | 6 | 1.4 |
| *Dero digitata* | 70 | 22.8 | 108 | 13.8 | 162 | 27.6 | 184 | 44.2 |
| Immature Tubificidae w/ hairs | 9 | 2.9 | 39 | 5.0 | 48 | 8.2 | 0 | - |
| *Aulodrilus pigueti* | 4 | 1.3 | 76 | 9.7 | 18 | 3.1 | 0 | - |
| *Ilyodrylus templetoni* | 10 | 3.3 | 40 | 5.1 | 3 | < 1 | 0 | - |
| *Slavina appendiculata* | 0 | - | 11 | 1.4 | 29 | 4.9 | 4 | < 1 |
| *Nais pardalis* | 2 | < 1 | 24 | 3.1 | 0 | - | 0 | - |
| *Haemonais waldvogeli* | 0 | - | 13 | 1.6 | 22 | 3.7 | 0 | - |
| *Eclipidrilus* sp. | 9 | 2.9 | 0 | - | 1 | < 1 | 0 | - |
| *Pristina synclites* | 0 | - | 0 | - | 9 | 1.5 | 163 | 39.2 |
| *Dero furcata* | 0 | - | 0 | - | 12 | 2.0 | 2 | < 1 |
| *Lumbriculus variegatus* | 23 | 7.5 | 0 | - | 0 | - | 0 | - |
| *Nais communis* | 5 | 1.6 | 0 | - | 0 | - | 0 | - |
| *Dero trifida* | 0 | - | 0 | - | 18 | 3.1 | 0 | - |
| *Stylaria lacustris* | 0 | - | 0 | - | 0 | - | 3 | < 1 |
| *Pristina bidentata* | 0 | - | 1 | < 1 | 0 | - | 0 | - |
| Total Number Collected | 306 | | 783 | | 588 | | 416 | |
| Mean (SD) | 17 ( 6.1) | | 43.5 (16.9) | | 32.7 (9.3) | | 23.1 (6.5) | |
| Total Number of Species | | 10 | | 10 | | 12 | | 7 |
| Mean Number of Species ± SD (Range) Per Core Sample | | 4.9 ± 1.7 (2 - 8) | | 5.3 ± 1.8 (3 - 9) | | 5.1 ± 1.5 (3 - 8) | | 5.3 ± 1.4 (3 - 8) |

[a] Site A - 0.5 km upstream from treatment plant - fish positive for *Eustrongylides* larvae.
[b] Site B - Sewage treatment plant outflow - fish positive for *Eustrongylides* larvae.
[c] Site C - 0.5 km downstream from sewage treatment plant - fish positive for *Eustrongylides* larvae.
[d] Site D - Outflow from Lake Alice - fish negative for *Eustrongylides* larvae.

*pigueti*, and *Ilyodrylus templetoni* were collected from all positive sites, comprising 247 of 1677 (14.7%) of *Pristina synclites* was common at site D, making up 163 of 416 (39%) collected, but observed at 1 positive site only (site C), making up 9 of 588 (1.5%). All other oligochaetes made up 177 of 2093 (8.4%) of specimens collected. The mean number of oligochaete species did not differ between sites ($F$ = 0.32, $P$ = 0.814, df = 71). Mean number of species (SD) per sample ($n$ = 18 per site) were: A = 4.9 (1.7), B = 5.3 (1.8), C = 5.1 (1.5), and D = 5.3 (1.4).

Physico-chemical Characteristics of Water

Water samples were collected from 4 study sites in the Lake Alice watershed. Each site was sampled a total of 13 times between fall, 1994 and summer, 1997. Ten parameters, including DO, pH, conductivity, temperature, IC, TOC, TC, TN, TP, and chlorophyll-a were analyzed and are presented in Table 17.

Mean DO was higher at site A (7.3 mg/l) than other sites ($P$ = 0.04). This site was dredged several times throughout the study, to remove deposited sediment, and was characterized by shallow water (< 0.5 m) with turbulent

Table 17. Physico-chemical characteristics of water collected from Lake Alice study sites 1994 - 1997. Samples per site ($n$ = 13). Significant differences (ANOVA) between sites are indicated.

| Parameter | Site A[a] $\bar{x} \pm SD$ | Site B[b] $\bar{x} \pm SD$ | Site C[c] $\bar{x} \pm SD$ | Site D[d] $\bar{x} \pm SD$ | Significance |
|---|---|---|---|---|---|
| Dissolved Oxygen (mg/l) | 7.3 ± 1.0 | 1.4 ± 0.8 | 2.1 ± 0.7 | 5.4 ± 1.4 | $F = 17.6$, $P < 0.001$, df = 51 |
| pH | 7.4 ± 0.3 | 7.2 ± 0.2 | 7.1 ± 0.1 | 7.4 ± 0.1 | $F = 1.1$, $P = 0.529$, df = 51 |
| Conductivity (μmhos/cm) | 269.7 ± 57.2 | 255.8 ± 59.6 | 259.6 ± 54.9 | 324.4 ± 76.3 | $F = 3.4$, $P = 0.260$, df = 51 |
| Temperature (C) | 23.6 ± 2.4 | 24.4 ± 3.2 | 24.4 ± 3.1 | 25.6 ± 6.9 | $F = 0.9$, $P = 0.471$, df = 51 |
| Inorganic Carbon (mg/l) | 23.6 ± 3.8 | 22.4 ± 3.2 | 24.4 ± 3.1 | 25.6 ± 3.7 | $F = 0.7$, $P = 0.579$, df = 51 |
| Organic Carbon (mg/l) | 7.2 ± 2.0 | 8.9 ± 2.9 | 8.7 ± 3.1 | 7.6 ± 2.6 | $F = 1.4$, $P = 0.246$, df = 51 |
| Total Carbon (mg/l) | 30.8 ± 3.9 | 31.4 ± 5.7 | 32.1 ± 8.2 | 33.1 ± 6.7 | $F = 0.6$, $P = 0.648$, df = 51 |
| Total Nitrogen (mg/l) | 1.4 ± 0.9 | 1.4 ± 0.8 | 1.2 ± 0.7 | 1.5 ± 0.4 | $F = 0.7$, $P = 0.236$, df = 51 |
| Total Phosphorus (mg/l) | 0.47 ± 0.28 | 1.12 ± 0.97 | 0.99 ± 0.75 | 0.67 ± 0.39 | $F = 2.7$, $P = 0.059$, df = 51 |
| Chlorophyll-a ($mg/m^3$) | 28.1 ± 14.0 | 124.5 ± 61.8 | 122.8 ± 57.4 | 40.5 ± 18.8 | $F = 18.2$, $P < 0.001$, df = 51 |

[a] Site A - 0.5 km upstream from treatment plant - fish positive for *Eustrongylides* larvae.
[b] Site B - Sewage treatment plant outflow - fish positive for *Eustrongylides* larvae.
[c] Site C - 0.5 km downstream from sewage treatment plant - fish positive for *Eustrongylides* larvae.
[d] Site D - Outflow from Lake Alice - fish negative for *Eustrongylides* larvae.

flow. Mean DO concentrations were lower at sites B (1.4 mg/l) and C (2.1 mg/l) than sites A or D (5.4 mg/l) ($P$ = 0.001). Stream flow appeared to be slow at sites B and C and water was often stagnant. Sites B and C had steep banks and thick vegetation, which blocked wind and probably reduced DO. Mosquitofish were abundant at sites B and C and often observed at the surface "gulping" air. Site D was located at the western end of Lake Alice and DO was probably influenced by wind fetch and wave action.

Dissolved oxygen results are presented for all sites (Fig. 15). Throughout the study, DO at site A declined from 8 mg/l in fall, 1994 to 5.9 mg/l in summer, 1997. Dissolved oxygen concentrations remained < 4 mg/l at sites B and C. Total nitrogen declined at all sites (Fig. 16).

Most mean water parameters, including pH, conductivity, temperature, IC, TOC, TC, and TN did not differ between sites (Table 17). Although not significant at $P < 0.05$, mean TP was almost significantly higher at site B than site D ($P = 0.059$).

Mean chlorophyll-a was higher at Sites B (124.5 mg/m$^3$) and C (122.8 mg/m$^3$) than sites A (28.1 mg/m$^3$) and D (40.5

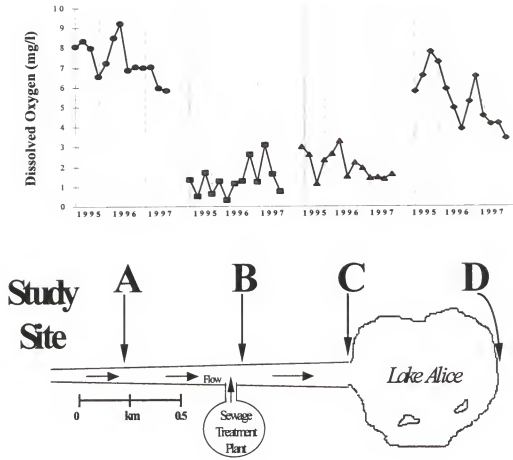


**Figure 15.** Dissolved oxygen concentrations at Lake Alice, Florida study sites, December 1994 - August 1997.

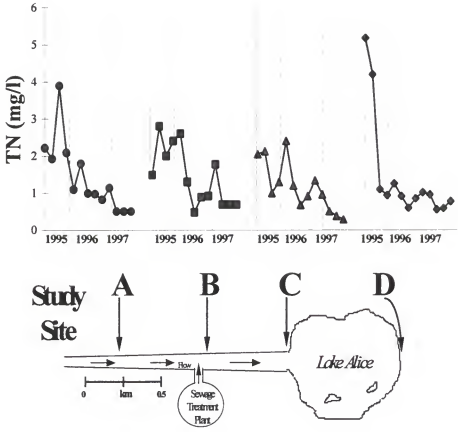


Figure 16. Total nitrogen concentrations at Lake Alice, Florida study sites, December 1994 - August 1997.

$mg/m^3$) ($P < 0.001$). Throughout the study, chlorophyll-a declined at sites B and C (Fig. 17). Algae blooms were observed at sites B and C during the summers of 1995 and 1996, but not in 1997.

Physico-chemical Characteristics of Sediment

Sediment samples were collected from 4 study sites in the Lake Alice watershed between December, 1994 and August, 1997. Three parameters, including TC, TN, and TP were analyzed and are presented in Table 18. Mean sediment TC was highest at site B (7.92 g/kg) and lowest at site D (2.31 g/kg) ($P < 0.001$). Sediment TC declined at all sites during early 1996 (Fig. 18). At positive sites (A, B, and C), TC increased in winter, 1996, then declined in 1997. At site D, TC increased in 1995, then declined throughout the duration of the study.

Mean sediment TN was higher at sites B and C than A and D ($P < 0.001$). Mean sediment TP was 14 times higher at site B than site D (Fig. 19) and approximately 10 times higher at sites A and C than Site D ($P < 0.001$). Although the treatment plant was retrofitted prior to the study, large amounts of phosphorus had been deposited into the sediment

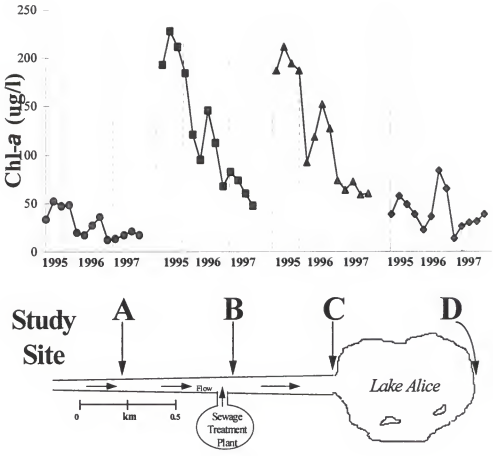


Figure 17. Chlorophyll-a concentrations at Lake Alice, Florida study sites, December 1994 - August 1997.

Table 18. Chemical characteristics of sediment collected from Lake Alice study sites 1994 - 1997. Means were compared between sites and significance is presented. Samples per site ($n = 13$).

| Parameter | Site A[a] $\bar{x} \pm SD$ | Site B[b] $\bar{x} \pm SD$ | Site C[c] $\bar{x} \pm SD$ | Site D[d] $\bar{x} \pm SD$ | Significance |
|---|---|---|---|---|---|
| Total Carbon (g/kg) | 5.57 ± 1.92 | 7.92 ± 3.06 | 5.08 ± 1.58 | 2.31 ± 1.99 | $F = 14.1$, $P < 0.001$, df = 51 |
| Total Nitrogen (g/kg) | 0.15 ± 0.06 | 0.61 ± 0.23 | 0.41± 0.10 | 0.24 ± 0.16 | $F = 18.8$, $P < 0.001$, df = 51 |
| Total Phosphorus (mg/kg) | 2915 ± 734 | 4078 ± 1215 | 2289 ± 984 | 289 ± 127 | $F = 43.6$, $P < 0.001$, df = 51 |

[a] Site A - 0.5 km upstream from treatment plant - fish positive for *Eustrongylides* larvae.
[b] Site B - Sewage treatment plant outflow - fish positive for *Eustrongylides* larvae.
[c] Site C - 0.5 km downstream from sewage treatment plant - fish positive for *Eustrongylides* larvae.
[d] Site D - Outflow from Lake Alice - fish negative for *Eustrongylides* larvae.

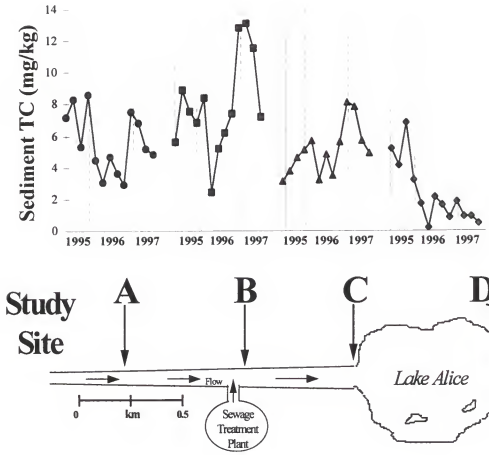


Figure 18. Sediment total carbon concentrations at Lake Alice, Florida study sites, December 1994 - August 1997.

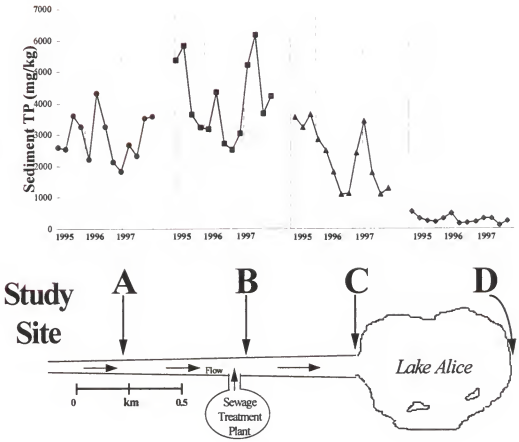


Figure 19. Sediment total phosphorus concentrations at Lake Alice, Florida study sites, December 1994 - August 1997.

and effects of the effluent plume were still measurable 3-years after wastewater was diverted. Sediment samples from sites B and C appeared to contain large amounts of particulate floc, while specimens from site D did not.

Sediment Grain Size

Sediment grain size and percent composition were collected and analyzed for study sites in the Lake Alice watershed (Table 19). Average grain sizes were 0.083 and 0.101 mm at sites B and C respectively and 0.050 mm at site D. Site D contained more sand (96.8%) than sites A (84.9%), B (90.6%), or C (89.8%). Percent composition of silt and clay was higher at sites A, B, and C than site D ($\chi^2 = 8.3$, $P = 0.0398$, df = 3).

Vegetation Survey

Mean percent area coverage (PAC) and percent vegetation composition was determined for each study site in the Lake Alice watershed, 1994 - 1997 (Table 20). Percent area coverage was highest at site D (31.4%) and lowest at A (12.5%) ($\chi^2 = 12.6$, $P = 0.0057$, df = 3). Site D was often covered with floating vegetation (water-lettuce and floating water-hyacinth), which was blown across the lake by wind.

Table 19. Sediment grain size analysis from Lake Alice study sites, Florida, 1996.

| Site | Percent Very Fine (< 0.1 mm) | Percent Fine (0.1 - 0.25 mm) | Percent Medium (0.25 - 0.5 mm) | Percent Coarse (0.5 - 1.0 mm) | Percent Very Coarse (> 1 mm) | Avg. Grain Size (mm) | Percent Sand (%) | Percent Silt (%) | Percent Clay (%) |
|---|---|---|---|---|---|---|---|---|---|
| A[a] | 5.8 | 32.0 | 50.2 | 9.4 | 2.6 | 0.071 | 84.9 | 3.9 | 11.2 |
| B[b] | 7.5 | 27.1 | 46.4 | 12.7 | 6.3 | 0.083 | 90.6 | 3.9 | 5.5 |
| C[c] | 5.7 | 11.3 | 49.0 | 28.4 | 5.6 | 0.101 | 89.8 | 4.7 | 5.5 |
| D[d] | 3.6 | 56.6 | 33.8 | 5.8 | 0.2 | 0.050 | 96.8 | 1.8 | 1.4 |

[a] Site A - 0.5 km upstream from treatment plant - fish positive for *Eustrongylides* larvae.
[b] Site B - Sewage treatment plant outflow - fish positive for *Eustrongylides* larvae.
[c] Site C - 0.5 km downstream from sewage treatment plant - fish positive for *Eustrongylides* larvae.
[d] Site D - Outflow from Lake Alice - fish negative for *Eustrongylides* larvae.

Table 20. Vegetation percent area coverage (PAC) and percent composition at Lake Alice, Florida study sites 1994 - 1997.

| Site (*n*) | % Coverage | % Floating | % Emergent | % Grasses | % Submersed |
|---|---|---|---|---|---|
| | $\bar{x}$ (SD) | $\bar{x}$ (SD) | $\bar{x}$ (SD) | $\bar{x}$ (SD) | $\bar{x}$ (SD) |
| A ($n = 18$) | 12.5 (4.9) | 3.5 (2.6) | 49 (5.7) | 45 (6.3) | 2.5 (2.2) |
| B ($n = 18$) | 16.8 (4.6) | 15.2 (4.4) | 44.5 (4.8) | 36.4 (6.7) | 3.9 (2.5) |
| C ($n = 18$) | 18.3 (4.8) | 11 (3.03) | 44.3 (8.1) | 41.4 (7.5) | 3.2 (2.2) |
| D ($n = 18$) | 31.4 (8.1) | 39.6 (6.7) | 30.1 (3.7) | 25.5 (6.1) | 4.9 (3.0) |

Percent floating vegetation was highest at site D ($\chi^2$ = 30.5, $P < 0.0001$, df = 3). The most common emergent plants at all sites were alligator weed, arrowheads, water pennywort, and pickerelweed. Percent emergent vegetation was higher at positive sites (A, B, and C) than site D ($\chi^2$ = 7.8, $P = 0.05$, df = 3). Grasses were common along the edge of the stream, especially after disturbances, such as mowing or dredging, and were often the first successional plants observed. Dominant grasses were barnyard grass, maidencane, and water paspalum (*Paspalum* spp.). Percent grasses was highest at site A and lowest at site D ($\chi^2$ = 9.25, $P$ = 0.0261, df = 3). Submerged plants were observed rarely in the Lake Alice watershed. Coontail was the most common submerged plant observed. There was no significant difference in the percent submerged vegetation between sites ($\chi^2$ = 0.91, $P$ = 0.82, df = 3).). Percent area coverage was highest at site D (31.4%) and lowest at A (12.5%) ($\chi^2$ = 12.6, $P$ = 0.0057, df = 3). Site D was often covered with floating vegetation (water-lettuce and floating water-hyacinth), which was blown across the lake by wind. Percent floating vegetation was highest at site D ($\chi^2$ = 30.5, $P <$ 0.0001, df = 3). The most common emergent plants at all

sites were alligator weed, arrowheads, water pennywort, and pickerelweed. Percent emergent vegetation was higher at positive sites (A, B, and C) than site D ($\chi^2 = 7.8$, $P = 0.05$, df = 3). Grasses were common along the edge of the stream, especially after disturbances, such as mowing or dredging, and were often the first successional plants observed. Dominant grasses were barnyard grass, maidencane, and water paspalum (*Paspalum* spp.). Percent grasses was highest at site A and lowest at site D ($\chi^2 = 9.25$, $P = 0.0261$, df = 3). Submerged plants were observed rarely in the Lake Alice watershed. Coontail was the most common submerged plant observed. There was no significant difference in the percent submerged vegetation between sites ($\chi^2 = 0.91$, $P = 0.82$, df = 3).

## Laboratory Results

### Morphology and Development of Eggs (22 C

*Eustrongylides ignotus* eggs were adhesive and stuck to most material contacted, including glass pipettes and wooden applicator sticks. Eggs were dislodged from plastic pipettes by gentle hydraulic flushing. Eggs ($n = 20$), fixed in hot glycerin and cleared in glycerin, measured mean ± SD,

(range) 72 ± 4 (69-77) $\mu$m long and 38 ± 1 (37- 40) $\mu$m wide. The shell was thick, mammillated, and characterized by multiple irregular pits (Fig. 20). Many of these pits contained either a single bacterium or small colonies of bacteria (Fig. 21).

Polar regions appeared blunt and more transparent than the main body of the egg. The eggs were amber and each contained one amorphous cell (Fig. 22).

One to 2-days after incubation in deionized (DI) water at 22 C, a dark band formed around the mid section of the egg. Three to 5-days after incubation, the cell began to cleave (Fig. 23) and at 15-days, 2 cells were clearly visible with 1 cell much darker than the other. By day-18, multiple cellular cleavage was observed and the embryo became indented on one side. The egg contained 1 motile larva at 21-days (Fig. 24). The larva moved infrequently (approximately once per minute). After 5-weeks, the larva did not move, but was clearly visible.

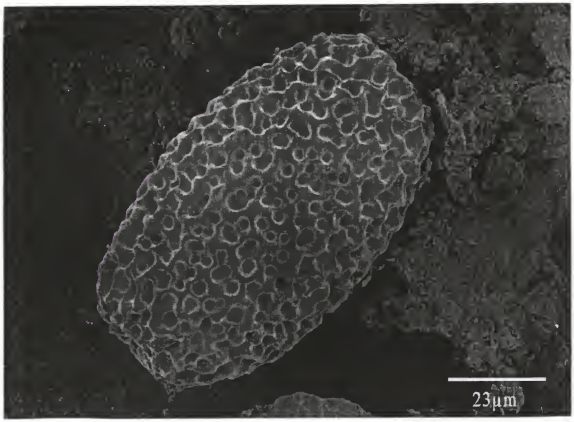


Figure 20. Scanning electron micrograph of egg of *Eustrongylides ignotus*. Note pitted shell.

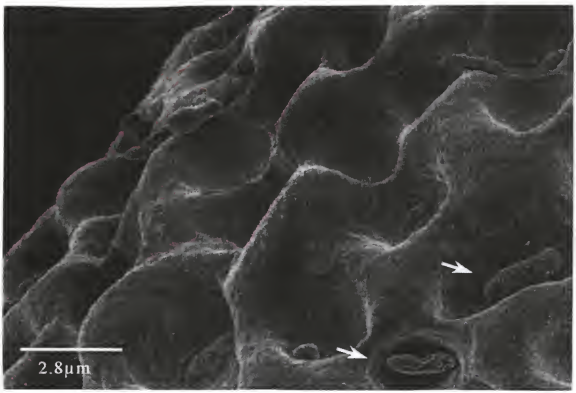


Figure 21. Scanning electron micrograph of eggshell of *Eustrongylides ignotus*. Note bacteria (arrows).

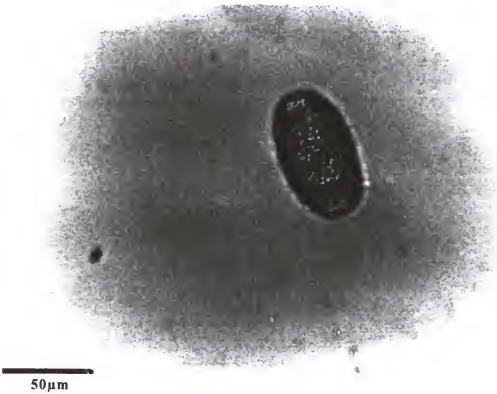


Figure 22. Micrograph of the egg of *Eustrongylides ignotus* at 1-day. Note one large cell.

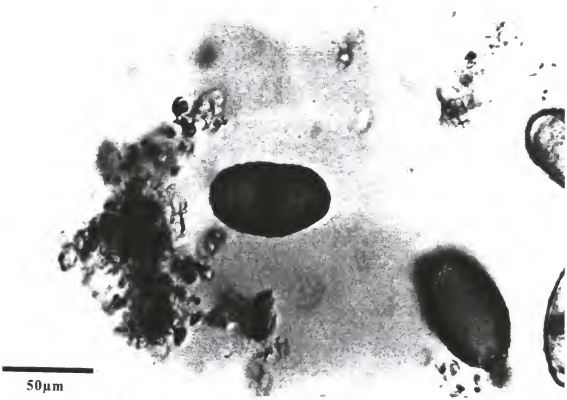


Figure 23. Micrograph of an egg of *Eustrongylides ignotus* at 5-days. Note dark band where cell begins to cleave.

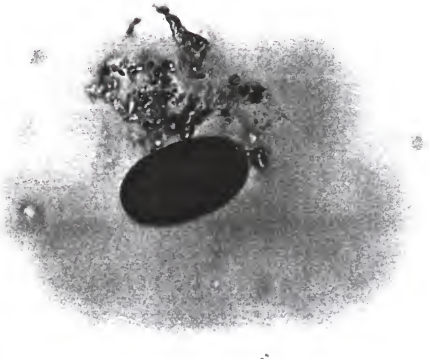


Figure 24. Micrograph of egg of *Eustrongylides ignotus* at 21-days. Note first-stage larva.

Development of Eggs at Various Temperatures

*Eustrongylides ignotus* eggs ($n$≈3,000) were separated into 15 cultures of 200 eggs each and incubated in DI water at 28, 24, and 19.5 C. Eggs were examined on a daily basis to determine development to the infective first-stage (L-1) (Table 21). Eggs contained motile first-stage larvae at 17, 22, and 28-days respectively. At 90-days, cultures incubated at 28 C had higher percent of developed eggs (61.4%) than those incubated at 24 C (40.8%) or 19.5 C (19.2%) ($\chi^2$ = 6.1, $P$ = 0.047, df = 2). During preliminary experiments, larvae developed at 30 C also, but water temperatures this high were observed infrequently in the field and this temperature regime was not included in experiments.

Motile first-stage larvae could be removed from the shell by incubating in pepsin (Fig. 25). Developed eggs were placed in DI water (replaced weekly) and stored at 4 and 19.5 C for ≤ 2-yrs. Eggs stored at 4 C remained infective to aquatic oligochaetes for 5-months, while those stored at 19.5 C remained viable for 2-yrs.

Table 21. Number of eggs containing larvae of *Eustrongylides ignotus* incubated at various temperatures, 1996.

| Incubation temperature (C) | First stage larva observed (days) | Mean percent of eggs[a] ± SD (range) containing larvae at 90-days |
|---|---|---|
| 28 | 17 | 61.4 ± 10.7 (44 - 68) |
| 24 | 22 | 40.8 ± 7.4 (32 - 51) |
| 19.5 | 28 | 19.2 ± 5.8 (11 - 26) |

[a]Five replicate cultures at each temperature.

Development of Eggs - Saline Conditions

*Eustrongylides ignotus* eggs were incubated in 4 saline concentrations and examined periodically for development to L-1 for 90-days (Table 22). Eggs did not develop in sea water, although some cellular cleavage was observed during the first week. Many eggs became crenated in sea water and several ruptured. Eggs incubated at lower saline concentrations developed normally, with no significant differences between treatments ($\chi^2 = 0.75$, $P = 0.688$, $df = 2$).


Figure 25. Micrograph of egg of *Eustrongylides ignotus* at 22-days. Note first-stage larva removed.

Table 22. Percent of eggs of *Eustrongylides ignotus* containing first-stage larva after 90-days incubation in various saline concentrations at 28 C.

| Salinity Parts Per Thousand (PPT) | Water Type[a] | Mean ± SD (range) percent of eggs with parasite larvae[b] |
|---|---|---|
| 40 | Hypersaline (sea water) | 0 |
| 20 | Mesosaline (brackish marsh) | 54.4 ± 14.9 (29 - 67) |
| 10 | Ground water runoff | 60.2 ± 16.7 (34 - 72) |
| 0 | Deionized water | 56.2 ± 17.2 (34 - 76) |

[a] Livingston 1984
[b] Five replicates at each concentration

Development of Eggs - Drydown Conditions

To simulate a drydown event, eggs were placed in petri dishes (aloquots of 100 eggs each) with sterile sand and DI water and exposed to direct sun for 28-days. At 28-days, treated eggs were resuspended in water and both groups were examined for parasite development (Table 23). Most of the water evaporated from the treatment group within 2-days. At 28-days, 3 of 482 (0.6%) eggs developed to L-1 in treatment cultures, while 286 of 491 (57.2%) developed in controls. At 60-days, 7 of 480 (1.5%) developed eggs were observed in treatment cultures and 294 of 472 (62.38%) were observed

in controls. Eggs were difficult to distinguish from sand and some were lost during the experiment. Many eggs adhered to grains of sand and were difficult to dislodge without destroying them.

Table 23. Percent of eggs containing larvae of *Eustrongylides ignotus* under drydown conditions. Cultures were exposed to direct sun during daylight hours and kept in a 28 C incubator at night. Daylight culture temperatures ranged (21 - 30 C). Cultures were examined at 28 and 60-days.

| Treatment[a] | Days incubated[b] | Mean ± SD (range) percent of eggs[c] with larvae |
|---|---|---|
| Control | 28 | 57.2 ± 14.5 (39 - 75) |
| Drydown | 28 | 0.6 ± 0.9 (0 - 2) |
| Control | 60 | 58.8 ± 12.4 (43 - 75) |
| Drydown | 60 | 1.4 ± 1.7 (0-4) |

[a] Control cultures were covered with glass and water was maintained at 1cm, drydown cultures were covered with 1 mm cheesecloth and water was allowed to evaporate.
[b] At 28-days, drydown cultures were resuspended in water and incubated at 28 C.
[c] Five replicates of each.

## Experimental Infection of Oligochaetes and Chironomids

Benthic invertebrates were collected from a sewage treatment plant outflow canal on the Univ. of Florida campus. This site was chosen because fishes infected with *E. ignotus* larvae were collected from the site during this

study. Over 700 oligochaetes and 500 *Chironomus* spp. were examined and none was found to be naturally infected. Eight oligochaetes species were identified, including *L. hoffmeisteri* 108 of 720 (15.1%), *D. digitata* 99 of 720 (13.8%), *A. pigueti* 70 of 720 (9.7%), *I. templetoni* 37 of 720 (5.1%), *N. pardalis* 22 of 720 (3.1%), *H. waldvogeli* 12 of 720 (1.7%), *S. appendiculata* 5 of 720 (< 1%), and *P. bidentata* 1 of 720 (< 1%). The remaining 366 of 720 (50.8%) oligochaetes, were sexually immature Tubificidae. Based on species abundance and following Brinkhurst (1986), most 326 of 720 (45.3%) immature Tubificidae without hair setae were probably *L. hoffmeisteri*. The chironomids were identified to genus.

During preliminary studies, oligochaetes and chironomids were seperated into cultures of ~100 each and fed mature *E. ignotus* eggs. The cultures were incubated at 28, 24, and 19.5 C respectively and examined at 7-day intervals for 9-months. Some of the oligochaetes died over the course of the experiment and all of the chironomids either emerged as adults or died. At 284-days post ingestion (PI), I detected 1 *L. hoffmeisteri*, incubated at 28 C, infected with a single third-stage *Eustrongylides*

larva. This oligochaete was probably infected much earliar, but overlooked on previous examinations. All other oligochaetes and chironomids were negative.

Experimental Infection of Oligochaetes at Various Temperatures

Over 1,700 mature oligochaetes were collected from the U.F. sewage treatment plant, examined, and determined to be negative for infection with larvae of *Eustrongylides*. Oligochaetes were fed mature eggs and incubated at 28 and 19.5 C respectively (Tables 24 and 25). Control cultures were incubated at both temperatures and remained negative throughout the experiment. Larvae of *Eustrongylides* (L-2) were observed first in oligochaetes at 4-wk PI (28 C) and 12-wk (19.5 C). All other larvae collected were L-3. *Limnodrilus hoffmeisteri* was the only species infected. Infected oligochaetes were more prevelent ($\chi^2 = 183$, $P < 0.0001$, df = 1) and observed earlier (4 weeks vs. 12 weeks) at 28 than at 19.5 C. Most infected oligochaetes contained one larva, although 1 specimen was infected with 3 L-3 (35-days PI, 28 C). The cephalic end of the parasite was always aligned with the anterior end of the oligochaete. The cephalic end of the larva was usually found near the gonads

Table 24. Number of oligochaetes infected with larvae[a] of *Eustrongylides ignotus* at weeks post-ingestion of eggs. Cultures were incubated at 28 C and infected oligochaetes were removed when detected.

| | No. Infected | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Week Examined** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** |
| *Dero digitata* (*n* = 200) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Limnodrilus hoffmeisteri* (*n* = 200) | 0 | 0 | 0 | **3** | **14** | **11** | **16** | **9** | **13** | **4** | **1** | 0 |
| *Ilyodrylus tempeltoni* (*n* = 150) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Aulodrilus pigueti* (*n* = 150) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Nais pardalis* (*n* = 150) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

[a]Larvae collected at 4-weeks were L-2, all others were L-3.

Table 25. Number of oligochaetes infected with larvae[a] of *Eustrongylides ignotus* at weeks post-ingestion of eggs. Cultures were incubated at 19.5 C and infected oligochaetes were removed when detected.

| | No. Infected | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Week Examined** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** |
| *Dero digitata* (*n* = 200) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Limnodrilus hoffmeisteri* (*n* = 200) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | **2** |
| *Ilyodrylus tempeltoni* (*n* = 150) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Aulodrilus pigueti* (*n* = 150) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Nais pardalis* (*n* = 150) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

[a] Larvae were L-2

of the oligochaete (segments X - XI). These segments were highly vascularized. Although most larvae were located in the ventral blood vessel of the oligochaetes, some appeared free in the coelomic cavity. When infected oligochaetes were detected they were removed and fed to fish, or preserved for reference specimens.

Second-Stage Larvae (L-2) in Oligochaetes

Second-stage larvae of *Eustrongylides* (L-2) were observed first in 3 *L. hoffmeisteri* at 28-days PI (28 C) and 2 *L. hoffmeisteri* at 84-days PI (19.5 C)(Figs. 26-27). Although not observed, the initial molt in oligochaetes (L-1 to L-2) probably took place rapidly (within days after ingestion of the egg). Multiple infections of oligochaetes with L-2 larvae were not detected. Larvae were aligned in the same orientation as the oligochaetes (anterior to anterior), with the mouth of the parasite located in either segment X or XI of the oligochaete (Fig. 28). Segments X and XI contained the reproductive organs of Tubificids, which were often developed poorly in infected oligochaetes. The larvae were coiled in the anterior half of the oligochaetes (segments X - XIV) and frequent movement of larvae was observed in living oligochaetes. Histologic exam


Figure 26. Scanning electron micrograph of cephalic extremity of second-stage larva of *Eustrongylides ignotus*. Note inner (I) and outer (O) circle cephalic papillae.

Figure 27. Scanning electron micrograph of caudal extremity of second-stage larva of *Eustrongylides ignotus*. Note striations of cuticle.

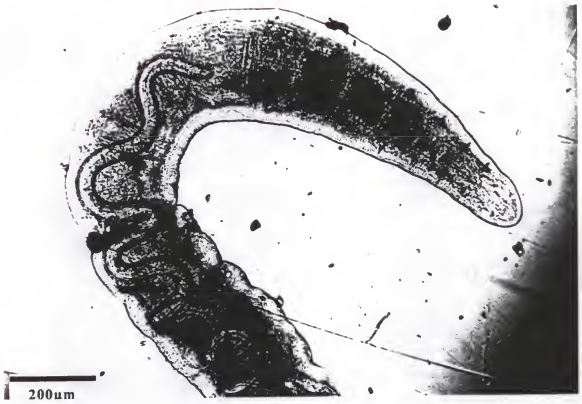


Figure 28. *Limnodrilus Hoffmeister* with second-stage larva of *Eustrongylides ignotus*. Note anterior extremity of larva is aligned toward the anterior end of oligochaete.

revealed that these larvae were located in the ventral blood vessel of the oligochaete (Fig. 29). These larvae were blunt at the anterior and posterior extremities and retained the cuticle of the first molt. Total length measured 605, 628, 692 μm (mean ± SD, 641.7 ± 45.1) respectively and 28, 35, 41 μm (34.7 ± 6.5) wide at the esophageal-intestinal junction. The esophagus was well defined and measured 246, 263, 302 μm (270.3 ± 28.7) in length. The buccal cavity was 12, 16, 18 μm (15.3 ± 3.1) long.

Third-Stage Larvae (L-3) in Oligochaetes

Third stage larvae were first detected in the ventral blood vessel of oligochaetes at 35-days PI (28° C). The second molt (L-2 to L-3) occurred between 28 and 35-days. Larvae were bi-colored, with the anterior portion (mouth to esophageal-intestinal junction) a pale cream color and the remainder of the worm bright red. The mouth of the parasite was always located in either segment X or XI with the remainder of the body coiled and extending as far posterior as segment XX. Histological changes were not observed in oligochaetes and although the larvae occupied a considerable


Figure 29. Histologic sagittal section of *Limnodrilus hoffmeisteri* with second-stage larva of *Eustrongylides ignotus*. Note larva is contained within the ventral blood vessel of the oligochaete.

amount of space in segments X - XX, the oligochaetes survived and appeared to function normally. One infected oligochaete survived in culture for 284-days post ingestion and contained a single third-stage larva. Most oligochaetes contained one larva only, although one specimen contained two larvae at 63-days PI. All larvae ($n$ = 68) collected between 35 and 77-days PI were third stage. Some larvae ($n$ = 16) were killed and mounted for description and voucher specimens, while all others were examined and then fed to fishes as part of another experiment.

Larvae retained the cuticles of the first and second molts, which were visible at anterior and posterior extremities. Major dimensions are given in Table 26. Female L-3 were significantly longer than males ($t$-test, $P$ = 0.042).

while those of the outer circle were broad based and had nipple-like apices. Several lateral field somatic papillae (3 - 5) were present between the inner and outer circle of labial papillae, while somatic papillae were located laterally and posterior to the outer circle. The posterior extremity of both genders had a terminal anus and the reproductive primordia was developed poorly.

Table 26. Major dimensions of third stage larvae of *Eustrongylides ignotus* from experimentally infected oligochaetes 35 - 77 days post infection. Results are mean (range).

| | Male | Female |
|---|---|---|
| Number (*n*) | 7 | 9 |
| Total length (mm) | 12.8 (7.5 - 16.8) | 14.9 (8.7 - 18.3) |
| Esophagus length (mm) | 4.2 (2.6 - 5.6) | 5.2 (3.1 - 7.1) |
| Rectal-intestinal junction to posterior extremity (μm) | 305.3 (224 - 412) | 352 (284 - 435) |
| Buccal cavity length (μm) | 82.3 (63 - 98) | 93.6 (72 - 109) |
| Width at esophageal-intestinal junction (μm) | 138.7 (117 - 151) | 141.9 (130 - 161) |

Several pilot experiments of oligochaetes fed eggs failed to produce infection. The eggs must be mature and contain L-1 before being consumed by the oligochaete. Undeveloped eggs were digested and passed through the oligochaetes within 2-days.

From the previous experiments, I determined the optimal experimental conditions for infecting *L. hoffmeisteri* with *E. ignotus*. I fed mature eggs to oligochaetes, incubated the oligochaete cultures at 28 C, and examined each specimen weekly under a dissecting light microscope. Using this technique, I infected > 200 oligochaetes for experimental transmission to fish.

Experimental Infection of Fishes

Transmission from infected oligochaetes (L-2)

Mosquitofish ($n$ = 31), warmouth ($n$ = 4), and largemouth bass ($n$ = 1) were each fed 1 oligochaete (*L. hoffmeisteri*) infected with a single second-stage *Eustrongylides* larva (26 - 35-days PI.). None of the experimental fish was infected when examined 14 - 45-days PI. In addition, control mosquitofish ($n$ = 30), warmouth ($n$ = 10), and largemouth bass ($n$ = 7) were kept in separate aquaria and examined on day-35. All control fishes were negative for larvae of *Eustrongylides*.

Transmission from infected oligochaetes (L-3

Large predator fishes, including warmouth ($n$ = 4) and largemouth bass ($n$ = 3) were each fed one infected oligochaete containing a third-stage larva (> 180-days PI). Fishes were killed and examined 14 to 30-days PI. All fishes were negative and no dead larvae or lesions resulting from parasite migration were observed.

Mosquitofish ($n$ = 168) were placed in 16 aquaria. Each aquarium contained a different combination of water type (sewage effluent, tap water), aquatic macrophytes (present, not present), sediment type (sterile treatment plant

sediment, sterile sand), and sediment slope (horizontal, 45° gradient). Each mosquitofish was fed 1 infected oligochaete (L-3) (> 180-days PI). All oligochaetes were consumed and none was regurgetated or found loose in the aquaria. Fish were killed and examined 30 - 205-days PI. Results are presented in Table 27.

One female mosquitofish (2.1 cm SL), examined 127 days PI, was infected with 1 L-3 *E. ignotus*. The larva was was encapsulated (capsule 5 mm diameter), attached to intestinal mesentary, and measured 27.9 mm total length. This mosquitofish did not contain fish eggs or embryos. Another female mosquitofish (2.3 cm SL), examined 184-days PI, contained 1 encapsulated larva. The cyst measured 9 mm in diameter and appeared to be attached to the ovary. When dissected, the cyst contained a coiled, bright red, fourth-stage larval *Eustrongylides*. The larva was a male and measured 8.7 cm in length. Again, this mosquitofish was not pregnant and no ova were observed.

All remaining mosquitofish were negative and no dead larvae or lesions were observed. Many of the female fish were pregnant and had multiple eggs and embryos. I was unable to determine which of the experimental conditions may

Table 27. Total number of mosquitofish (*Gambusia holbrooki*) fed oligochaetes infected with L-3 *Eustrongylides ignotus*. Fish were killed and examined 90 - 205-days post ingestion (PI) for larval *E. ignotus*. All fish were kept at 26 C with control fish ($n$ = 32 - 41) for each treatment. All controls were negative.

| Treatment | Water Type[a] | Aquatic Vegetation[b] | Sediment Type[c] | Sediment Sloped[d] | Number Examined | Number Infected | Percent (%) Infected |
|---|---|---|---|---|---|---|---|
| 1 | S | P | SS | H | 7 | 0 | - |
| 2 | S | P | SS | G | 9 | 1[e] | 11.1 |
| 3 | S | P | TP | H | 11 | 0 | - |
| 4 | S | P | TP | G | 14 | 0 | - |
| 5 | S | NP | TP | H | 9 | 0 | - |
| 6 | S | NP | TP | G | 12 | 0 | - |
| 7 | S | NP | SS | H | 5 | 0 | - |
| 8 | S | NP | SS | G | 8 | 0 | - |
| 9 | T | P | SS | H | 11 | 0 | - |
| 10 | T | P | SS | G | 12 | 0 | - |
| 11 | T | P | TP | H | 15 | 0 | - |
| 12 | T | NP | SS | G | 14 | 1[f] | 7.1 |
| 13 | T | NP | TP | H | 13 | 0 | - |
| 14 | T | NP | TP | G | 11 | 0 | - |
| 15 | T | NP | SS | H | 8 | 0 | - |
| 16 | T | P | TP | G | 9 | 0 | - |
| | | | | Total | 168 | 2 | 1.2 |

[a] Water type: S = sewage effluent, T = tap water
[b] Aquatic macrophytes: P = present, NP = not present
[c] Sediment type: TP = treatment plant sediment (sterile), SS = sterile sand
[d] Sediment slope: G = 45° gradient, H = horizontal gradient
[e] L-4: 184-days PI in female mosquitofish
[f] L-3: 127-days PI in female mosquitofish

influence transmission because of the low prevalence of infected fish. Although sterile sand was present in aquaria containing infected fish, water type, aquatic macrophytes, and sediment slope were different for each.

Fishes were difficult to infect with oligochaetes (< 1% transmission). Based on these data, *E. ignotus* larvae, transmitted from oligochaetes to fish, molted from L-3 to L-4 between 127 - 184-days PI.

Direct transmission from eggs

I explored alternative methods of parasite transmission, including feeding mature eggs and injecting eggs into the stomachs of fishes. I observed that eggs of *E. ignotus* adhered to commercial flake fish food when mixed in aqueous solution. In a pilot study, mosquitofish ($n$ = 142) were fed a mixture of flake fish food and eggs of *E. ignotus*. The fish were transferred to 5 aerated aquaria containing sterile sand with tap water and maintained at room temperature (~22 C). Control mosquitofish ($n$ = 32) were placed in a sixth aquarium under the same conditions. Some fish died during the experiment (experimental - $n$ = 37, control - $n$ = 8). Fish were killed and examined at 124-days PI ($n$ = 30) and 276-days PI ($n$ = 75). One male fish

(1.8 cm SL, killed 124 days PI) contained a pale pink 15.2 mm larva (L-3), which was coiled, encapsulated, and attached to the intestinal mesentery. This cyst measured 4.2 mm in diameter. A female fish (killed 276-days PI) contained a bright red cyst (11 mm diameter), which was attached to intestinal mesentary (Fig. 30). Upon dissection, a 4.6 cm L-4 male *Eustrongylides* larva was observed (Fig. 31). The control fish were killed and examined at 276-days PI and all were negative.

This experiment was repeated with different treatments to each of 16 aquaria. Treatments included variations in water type, aquatic macrophytes, sediment type, and sediment slope. Mosquitofish were fed eggs and examined at 163 to 171-days PI (Table 28). Infected fish ($n$ = 14) were detected in 7 / 16 (44%) aquaria and under all treatment types. All infected fish contained a single fourth-stage larva. Prevalences of infected fish were not influenced by treatment ($\chi 2$ = 11.98, df = 7, P = 0.101).

Replicate time trials were conducted to determine the time required for development of parasite larvae in mosquitofish (Table 29). Parasite eggs were injected into the oral cavity to insure inoculation. Larval

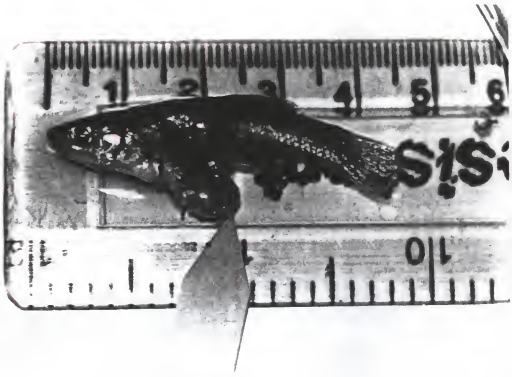

Figure 30. *Gambusia Holbrooki* with cyst containing fourth-stage larva of *Eustrongylides ignotus*. Fish was fed infective eggs and examined 276-days PI.


Figure 31. *Gambusia holbrooki* with fourth-stage larva (arrow) of *Eustrongylides ignotus*. Fish was fed infective eggs and examined 276-days PI. Scale bar is in inches.

Table 28. Total number of mosquitofish (*Gambusia holbrooki*) fed infective eggs and examined 163 - 171-days post infection (PI) for larval *Eustrongylides ignotus*. All fish were kept at 26 C with control fish ($n = 30$) for each treatment. All Larvae were fourth-stage (L-4). All controls were negative.

| Treatment | Water Type[a] | Aquatic Vegetation[b] | Sediment Type[c] | Sediment Slope[d] | Number Examined | Number Infected | Percent (%) Infected |
|---|---|---|---|---|---|---|---|
| 1 | S | P | SS | H | 27 | 0 | - |
| 2 | S | P | SS | G | 21 | 0 | - |
| 3 | S | P | TP | H | 18 | 3 | 16.7 |
| 4 | S | P | TP | G | 26 | 2 | 7.7 |
| 5 | S | NP | TP | H | 30 | 0 | - |
| 6 | S | NP | TP | G | 22 | 0 | - |
| 7 | S | NP | SS | H | 30 | 1 | 3.3 |
| 8 | S | NP | SS | G | 19 | 0 | - |
| 9 | T | P | SS | H | 28 | 4 | 14.3 |
| 10 | T | P | SS | G | 29 | 0 | - |
| 11 | T | P | TP | H | 16 | 1 | 6.3 |
| 12 | T | NP | SS | G | 30 | 0 | - |
| 13 | T | NP | TP | H | 23 | 0 | - |
| 14 | T | NP | TP | G | 27 | 0 | - |
| 15 | T | NP | SS | H | 19 | 1 | 5.3 |
| 16 | T | P | TP | G | 24 | 2 | 8.3 |
| | | | | Total | 389 | 14 | 3.6 |

[a] Water type: S = sewage effluent, DI = deionized water
[b] Aquatic vegetation: P = present, NP = not present
[c] Sediment type: TP = treatment plant sediment, SS = sterile sand
[d] Sediment slope: G = 45° gradient, H = horizontal gradient

Table 29. Total number and gender of fish (*Gambusia holbrooki*) infected, larval stage of development, and location in fish of *Eustrongylides* larvae. Eggs containing larval *Eustrongylides* ($n \approx 10$ / inoculum) were injected into the oral cavity of each fish. All larvae detected ≥ 56-days were encapsulated. Each aquarium (experimental - $n = 8$, control - $n = 2$) contained 30 fish (15 males and 15 females), sterile sand, and tap water at 26 C. One fish / aquarium was killed and examined each week from 2 - 32-weeks post ingestion. All controls were negative.

| No. Days Post Infection | No. Fish Infected | Larval Stage[a] | Location in Fish[b] | Gender of Fish |
|---|---|---|---|---|
| 35 | 1 | L-3 (L) | RT | Female |
| 56 | 1 | L-3 (E) | IT | Male |
| 77 | 1 | L-3 (E) | RT | Male |
| 84 | 1 | L-3 (E) | CC | Female |
| 105 | 3 | L-4 (E) | RT(1), CC(2) | 2 Females, 1 Male |
| 126 | 2 | L-4 (E) | CC | 2 Females |
| 154 | 1[c] | L-4, L-4 (E) | CC | Male |
| 182 | 1 | L-4 (E) | CC | Male |

[a]E = encapsulated, L = loose

[b]Location in fish: RT = reproductive tract, IT = intestinal mesentery, CC = coelomic cavity

[c]This fish was infected with 2 larvae, encapsulated in the same cyst

*Eustrongylides* were identified from 11 of 240 (4.6%) experimental fish. Male and female mosquitofish were susceptible to infection. Although L-2 were not detected and probably missed during examination, 1 L-3 was not encapsulated and was attached to the ovary of a female mosquitofish at 35-days PI. All larvae collected 35 to 84-days PI were L-3. Development from L-3 to L-4 occurred 84 - 105-days PI. All larvae collected 56-days PI were red and encapsulated. Most larvae were single infections, but 1 male fish, examined at 154-days PI, contained 2 fourth-stage larvae intertwined and encapsulated in one cyst. Most larvae were collected from the coelomic cavities or reproductive tracts of mosquitofish, although 1 L-3 was attached to the intestinal mesentary.

Major dimensions of third-stage larvae from fish

All third-stage larvae collected from fish retained the second-stage cuticle but the first-stage cuticle was not observed. The cephalic extremities were conical and cephalic papillae were well defined (Fig. 32). The caudal extremity of a third-stage larva, with terminal opening, is shown in Fig. 33. The genital primordium of both genders was well developed. Major dimensions of L-3 from naturally

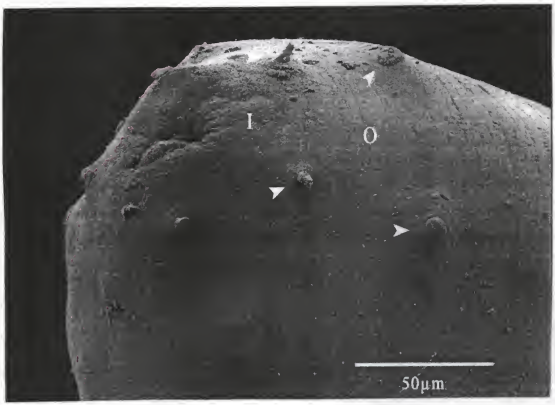


Figure 32. Scanning electron micrograph of cephalic extremity of third-stage larva of *Eustrongylides ignotus*. Note inner circle (I) and outer circle (O) papillae. Larva was obtained from *Gambusia holbrooki* fed eggs at 84-days PI.

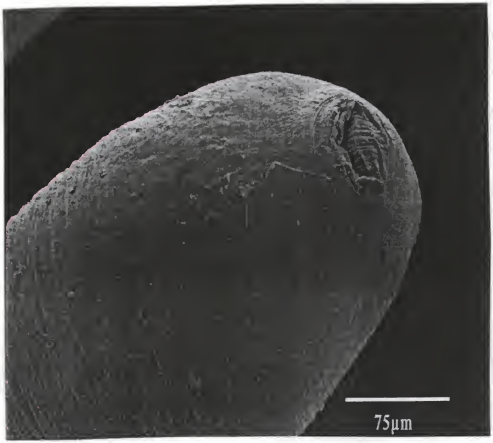


Figure 33. Scanning electron micrograph of caudal extremity of third-stage larva of *Eustrongylides ignotus*. Note terminal opening. Larva was obtained from *Gambusia holbrooki* fed eggs at 84-days PI.

infected fish are presented (Table 30). Mean total lengths of female and male larvae, collected from naturally infected fish, did not differ significantly ($t = 0.59$, $P = 0.28$, df = 20). Although 1 female L-3 only was collected from fish-fed oligochaetes, total length measured 15.3 mm and was within the range of those collected from naturally infected fish. Major dimensions of third-stage larvae collected from experimental fish fed eggs are given in Table 31. Total lengths were similar to those observed in naturally infected fish.

Table 30. Major dimensions of third-stage *Eustrongylides ignotus* collected from naturally infected mosquitofish (*Gambusia holbrooki*) from Florida. Results are mean ± SD (range).

| | Male | Female |
|---|---|---|
| Number (*n*) | 10 | 12 |
| Total length (mm) | 23.6 ± 3.7<br>(16.1 - 23.8) | 24.9 ± 6.1<br>(14.6 - 34.5) |
| Esophagus length (mm) | 7.5 ± 0.5<br>(6.3 - 7.9) | 8.2 ± 1.1<br>(6.5 - 10.3) |
| Rectal-intestinal junction to posterior extremity (μm) | 342 ± 84<br>(246 - 468) | 339 ± 82<br>(241 - 518) |
| Bucal cavity length (μm) | 85.9 ± 7.1<br>(76.2 - 102.3) | 95.2 ± 10.9<br>(76.3 - 112) |
| Width at esophageal-intestinal junction (μm) | 229 ± 43.6<br>(167 - 290) | 271 ± 47.7<br>(180 - 420) |

Table 31. Major dimensions of third-stage larvae of *Eustrongylides ignotus* from experimental mosquitofish (*Gambusia holbrooki*) fed infective eggs.

| | Female |
|---|---|
| Number (*n*) | 3 |
| Total length (mm) | 26.7 ± 12.6 (12.4 -36.3) |
| Esophagus length (mm) | 8.5 ± 2.5 (5.9 - 10.8) |
| Rectal-intestinal junction to posterior extreamity (μm) | 378 ± 120 (240 - 455) |
| Bucal cavity length (μm) | 104 ± 26 (76 - 126) |
| Width at esophageal-intestinal junction (μm) | 348 ± 162 (162 - 460) |

Major dimensions of fourth-stage larvae from fish

Fourth-stage larvae retained the cuticles of the second and third-stage, which were visible at the anterior and posterior extremities (Figs 34 -37). The anterior third of the larva was pinkish to red while the remainder of the worm was bright red. Cephalic papillae of fourth-stage larvae were larger and more well developed than those in third-stage larvae. The genital primordium was well developed.

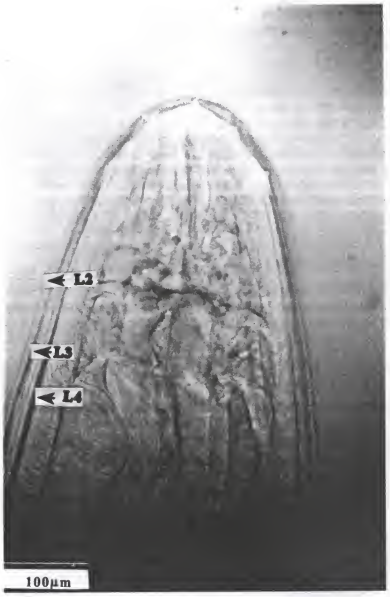


Figure 34. Cephalic extremity of male fourth-stage larva of *Eustrongylides ignotus*. Note cuticles retained from previous molts. Larva was obtained from a *Gambusia holbrooki* fed infective eggs and examined 154-days PI.

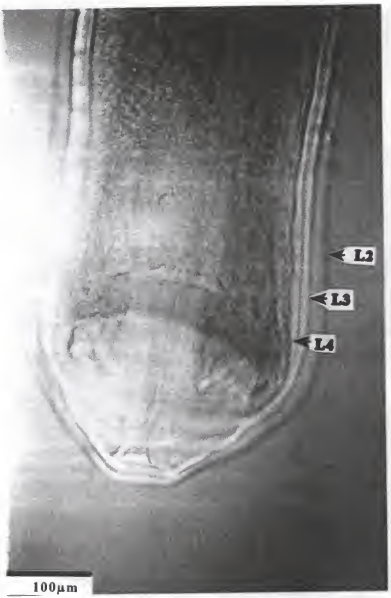


Figure 35. Caudal extremity of male fourth-stage larva of *Eustrongylides ignotus*. Note cuticles retained from previous molts. Larva was obtained from a *Gambusia holbrooki* fed infective eggs and examined 154-days PI.

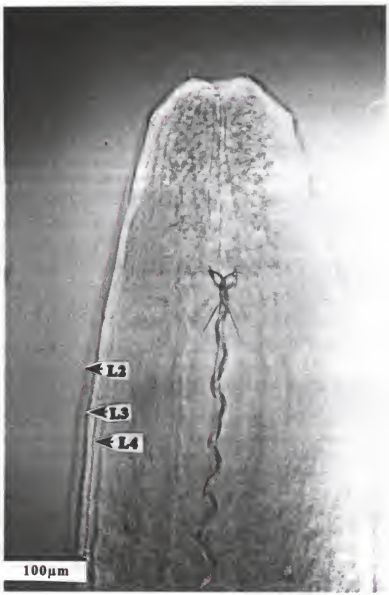


Figure 36. Cephalic extremity of female fourth-stage larva of *Eustrongylides ignotus*. Note cuticles retained from previous molts. Larva was obtained from a *Gambusia holbrooki* fed infective eggs and examined 126-days PI.

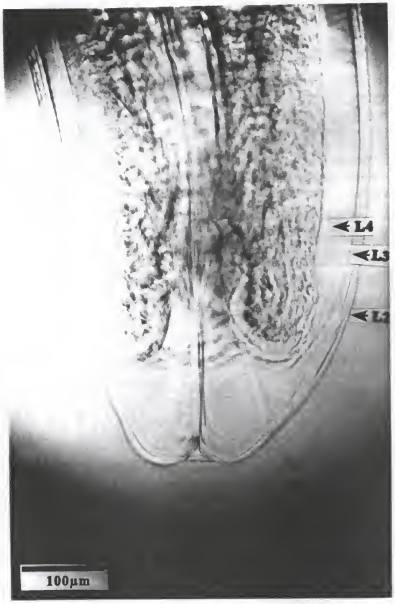


Figure 37. Caudal extremity of female fourth-stage larva of *Eustrongylides ignotus*. Note cuticles retained from previous molts. Larva was obtained from a *Gambusia holbrooki* fed infective eggs and examined 126-days PI.

Major dimensions of L-4 from naturally infected fish are given in Table 32. Fourth-stage larvae were larger than third-stage larvae collected from naturally infected fish (male: $t = 10.4$, $P < 0.0001$, df = 18; female: $t = 17.9$, $P < 0.001$, df = 20). Although 1 female L-4 only was collected from fish fed infected oligochaetes (184-days PI), appearance and total length (89 mm) was similar to those of L-4 collected from naturally infected fish. Female L-4, collected from naturally infected fish were larger (total length) than males ($t = 3.2$, $P = 0.002$, df = 18).

Table 32. Major dimensions of fourth-stage larvae of *Eustrongylides ignotus* from naturally infected mosquitofish (*Gambusia holbrooki*) from Florida. Results are mean ± SD (range).

| | Male | Female |
|---|---|---|
| Number (*n*) | 10 | 10 |
| Total length (mm) | 83.8 ± 17.9<br>(62.8 - 111.6) | 107 ± 14.4<br>(86.4 - 126.7) |
| Esophagus length (mm) | 17 ± 5.4<br>(9.8 - 23.3) | 21.7 ± 6.2<br>(14.8 - 30.3) |
| Rectal-intestinal junction to posterior extreamity (μm) | 728 ± 120<br>(590 - 920) | 903 ± 208<br>(612 - 1246) |
| Bucal cavity length (μm) | 171 ± 36.4<br>(105 - 210) | 206 ± 52.6<br>(136 - 264) |
| Width at esophageal-intestinal junction (μm) | 397 ± 24.9<br>(352 - 426) | 460 ± 47.7<br>(408 - 521) |

Major dimensions of fourth-stage larvae from mosquitofish fed infective eggs are given in Table 33. Female L-4 from naturally infected fish were larger (total length) than females from experimental fish fed eggs ($t$ = 2.8, $P$ = 0.015, df =13), although other physical characteristics such as color, size and arrangement of cephalic papillae, and development of genital primordia were similar.

Table 33. Major dimensions of fourth-stage *Eustrongylides ignotus* larvae collected from experimental mosquitofish (*Gambusia holbrooki*) fed infective eggs.

| Parasite gender | Male | Female |
|---|---|---|
| Number (*n*) | 3 | 6 |
| Total length (mm) | 73 ± 12.5 (61 - 86) | 92.8 ± 5.5 (85 - 98) |
| Esophagus length (mm) | 17.7 ± 0.6 (17 -18) | 19.8 ± 0.8 (19 - 21) |
| Rectal-intestinal junction to posterior extreamity (μm) | 678 ± 59.4 (610 - 720) | 749 ± 50.6 (690 - 806) |
| Bucal cavity length (μm) | 166.3 ± 47.6 (112 - 201) | 478.2 ± 344.8 (143 - 806) |
| Width at esophageal-intestinal junction (μm) | 306.7 ± 104 (200 - 408) | 475 ± 11.7 (460 - 488) |

Cephalic papillae of fourth-stage larvae were well developed, with inner circle papillae having large pointed apaces and outer circle papillae with nipple-like apaces. Lateral field somatic labial papillae were clearly visible between inner and outer circle papillae. (Figs. 38-40). Terminal opening of caudal extremity was clearly visible (Fig. 41).

A histologic cross-section of a mosquitofish infected with a fourth-stage larva of *Eustronglides ignotus* was performed (Fig.42).

Experimental transmission to paratenic hosts (fishes)

Mosquitofish, each infected with 1 advanced-stage *Eustrongylides* larva, were fed to black crappie ($n$ = 3), largemouth bass ($n$ = 4), and warmouth ($n$ = 4). All fishes were killed 30-days PI, dissected, and examined (Table 34). All control fishes ($n$ = 32) were negative.

Infected mosquitofish were ingested intact, although body parts such as fins and tissue were expelled occasionally from the mouth and gills of predators. None of the worms was regurgitated and no parasites were found in

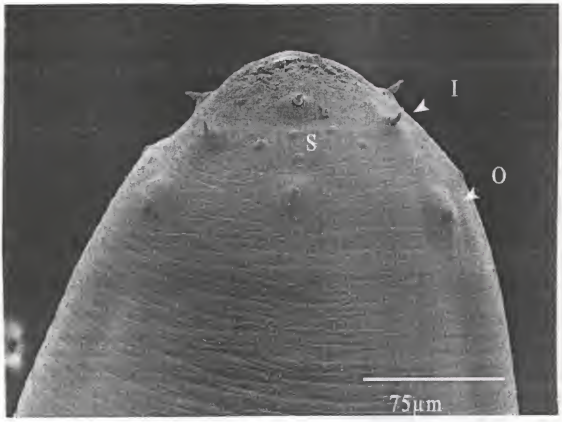


Figure 38. Scanning electron micrograph of cephalic extremity of fourth-stage larva of *Eustrongylides ignotus*. Note inner circle (I), latteral field somatic papillae (S), and outer circle papillae (O). Larva was obtained from *Gambusia holbrooki* fed infective eggs and examined 182-days PI.

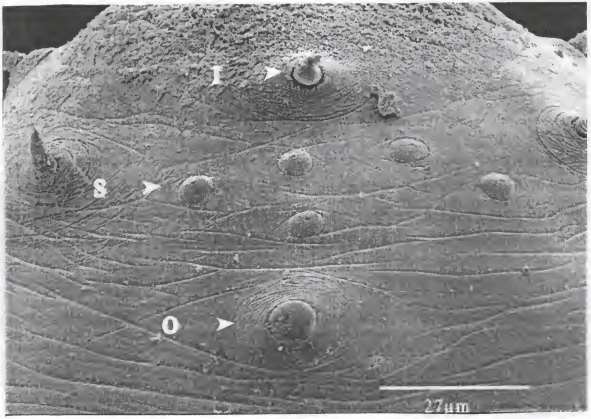


Figure 39. Scanning electron micrograph of cephalic extremity of fourth-stage larva of *Eustrongylides ignotus*. Note inner circle (I), latteral field somatic papillae (S), and outer circle papillae (O). Larva was obtained from *Gambusia holbrooki* fed infective eggs and examined 182-days PI.

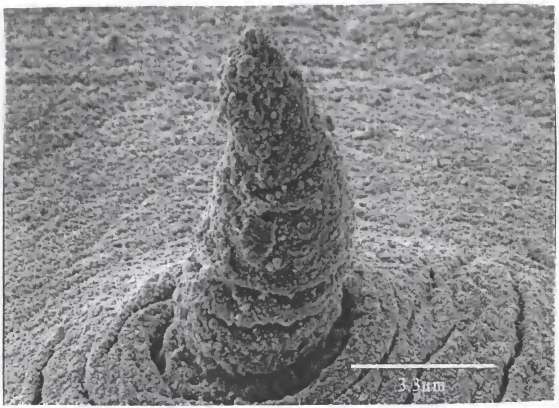


Figure 40. Scanning electron micrograph of inner circle cephalic papilla of *Eustrongylides ignotus*. Larva was obtained from *Gambusia holbrooki* fed infective eggs and examined 182-days PI.

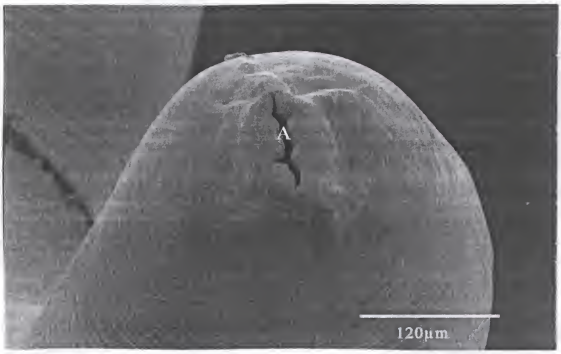


Figure 41. Scanning electron micrograph of caudal extremity of fourth-stage larva of *Eustrongylides ignotus*. Note terminal anus (A). Larva was obtained from *Gambusia holbrooki* fed infective eggs and examined 182-days PI.

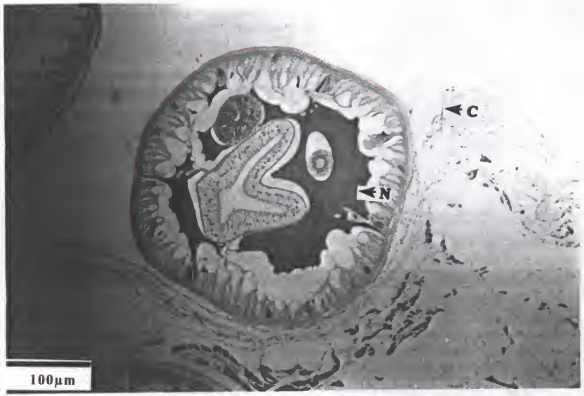


Figure 42. Histoligic cross section of *Gambusia holbrooki*. Note fourth-stage nematode larva of *Eustrongylides ignotus* (N). Cyst wall is shown (C).

Table 34. Number and location of fourth-stage *Eustrongylides* larvae recovered from paratenic hosts 30-days post ingestion of naturally infected *Gambusia holbrooki*.

| Host species | No. larvae ingested | No. larvae recovered | Location in host[a] |
|---|---|---|---|
| *Lepomis gulosus* | 1 | 0 | - |
| *Lepomis gulosus* | 1 | 1 | I |
| *Lepomis gulosus* | 2 | 1 | CC |
| *Lepomis gulosus* | 2 | 2 | DM, K |
| *Micropterus salmoides* | 1 | 1 | CC |
| *Micropterus salmoides* | 2 | 2 | IM, SB |
| *Micropterus salmoides* | 2 | 1 | CC |
| *Micropterus salmoides* | 3 | 3 | CC, DM, SB |
| *Pomoxis nigromaculatus* | 1 | 1 | L |
| *Pomoxis nigromaculatus* | 3 | 3 | CC, DM, S |
| *Pomoxis nigromaculatus* | 4 | 2 | CC, K, |

[a]Location: BC = brain case, CC = coelomic cavity, DM = dorsal musculature, IM = intestinal mesentery, K = kidney, L = liver, S = spleen, SB = swim bladder.

the aquaria. Of the 22 parasites ingested, 17 (77%) live fourth-stage larvae were recovered from predators. No dead larvae were observed in fishes. Five larvae were not recovered and were probably digested. Larvae were recovered from all treated black crappie (Fig. 43) and largemouth bass, and 3 of 4 (75%) warmouth. None of the larvae was encapsulated but many were coiled, especially those found in

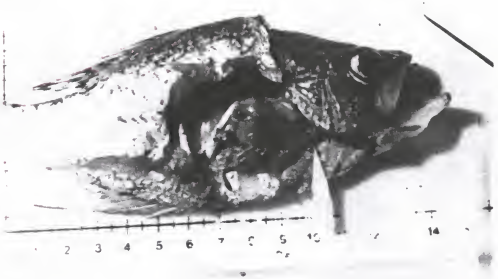

Figure 43. Black crappie (*Pomoxis nigromaculatus*) with 1 fourth-stage larva of *Eustrongylides ignotus*. This crappie was fed 1 *Gambusia holbrooki* infected with *Eustrongylides*.

the dorsal musculature. The most common location of parasites in the predators was the anterior portion of the coelomic cavity 6 of 17 (35%). Some larvae penetrated organs, such as the kidney, liver, spleen, and swim bladder. Gross lesions, with some hemorrhage, were observed along migration tracts. One larva was collected from the right lateral portion of the brain case of a black crappie and may have begun to migrate into the optic nerve.

Mean (SD) total lengths of L-4 from predators were: male ($n$ = 5) 83.1 (16.2), female ($n$ = 12) 103.7 mm (11.6). Although fourth-stage larvae in paratenic hosts were not encapsulated, they had similar morphology to those collected from naturally infected fish, which were encysted. There were no significant differences in total lengths between larvae from paratenic hosts and those from naturally infected fishes: male larvae ($t$ = 0.06, $P$ = 0.477, df = 13), female larvae ($t$ = 0.51, $P$ = 0.307, df = 20).

Behavioral Changes in Infected Fishes

I examined predation susceptibility of infected mosquitofish to three species of predatory fishes, including juvenile largemouth bass ($n$ = 6), warmouth ($n$ = 4), and bluegill ($n$ = 2). A 55-gallon aquarium with removable

plexiglass divider and remote observation windows was constructed. Predatory fish were allowed to acclimate to one half of the tank, while one infected and one uninfected mosquitofish (same gender and size) were placed in the other. The divider was removed and an observer recorded the number of capture attempts and time required for capture. Trials were repeated between 1 to 6 times for each predator. Predators were observed for behavioral alterations 4-days post ingestion of infected mosquitofish, then killed and necropsied.

Over a 3-month period, 38 trials were performed (Table 35). Infected mosquitofish were selected before non-infected fish in 31 of 38 (82%) of trials. To avoid predation, mosquitofish attempted to remain motionless and conceal themselves in vegetation. Infected mosquitofish appeared to move erratically and often left the cover of vegetation. Bluegill, largemouth bass, and warmouth are sight predators and appeared to select prey based on movement.

The mean number of capture attempts was greater for non-infected mosquitofish than infected fish ($t = 2.91$, $P = 0.0024$, $df = 74$). In addition, mean time of capture was

greater for non-infected than infected prey ($t = 2.82$, $P = 0.0031$, df = 74). When a capture attempt was made, the mosquitofish attempted to out-maneuver the predator and seek refuge in other patches of vegetation. Infected fish were often captured on the initial attempt and appeared unable to maneuver or swim as fast as non-infected fish.

Table 35. Number of times mosquitofish (*Gambusia holbrooki*) selected first, number of capture attempts, and time until capture by predators. Predators included juvenile largemouth bass (*Micropterus salminoides*) ($n = 6$), warmouth (*Lepomis gulosus*) ($n = 4$), and bluegill (*Lepomis macrochirus*) ($n = 2$). Infected mosquitofish contained advanced-stage *Eustrongylides* larvae, non-infected mosquitofish appeared normal. During each trial, predators were presented with a choice between 1 infected and 1 non-infected mosquitofish. Number of trials ( $n = 38$).

| | Infected | Non-infected |
|---|---|---|
| No. times (%) prey selected first | 31<br>(81.6) | 7<br>(18.4) |
| Mean ± SE (range) no. capture attempts | 2.7 ± 0.2<br>(1 - 6) | 3.9 ± 0.4<br>(1 - 13) |
| Mean ± SE (range) time until capture (min) | 12.4 ± 1.6<br>(3.3 - 47.8) | 21.7 ± 2.9<br>(4.3 - 60) |

During the 4-days post-ingestion of infected mosquitofish, aberrant behavior was observed in 8 of 12 (67%) of predators (Table 36). These behaviors were not observed in control fishes and included lethargy, buoyancy

abnormalities, loss of appetite, and convulsions. Lethargy was observed in 6 of 12 (50%) and buoyancy abnormalities in 5 of 12 (42%) of predators. Several of these fish remained motionless near the surface or suspended vertically in the water column and were caught easily by hand. Three of 12 (25%) predators had loss of appetite and refused to eat when offered non-infected immobilized prey. Convulsions, including erratic head shaking and full body tremors, were observed in 3 of 12 (25%) of predators. Most convulsions were observed within 3-hours PI.

At necropsy, larvae of *Eustrongylides* were collected from all predators, although some larvae ($n$ = 9) were not recovered from those predators receiving multiple infections (Table 36). Dead nematodes were not observed and missing larvae were probably digested. All larvae were L-4, motile, and not encapsulated. Larvae were identified in the coelomic cavity, dorsal musculature, and viscera. Most larvae, 16 of 29 (55.2%), were collected from the coelomic cavity in 11 of 12 (91.7%) of predators. Location of remaining larvae were in the intestinal mesentery, 6 of 29 (20.7%); dorsal musculature, 2 of 29 (6.9%); kidney, 2 of 29

Table 36. Number and location of parasites recovered 4-days post ingestion (PI), and abnormal behavior observations of predatory fishes after ingestion of mosquitofish (*Gambusia holbrooki*) infected with advanced stage larvae of *Eustrongylides*.

| Predator species | No. mosquitofish ingested | No. larvae recovered 4-days PI | Location of larvae in predator[a] | Abnormal behavior observed[b] |
|---|---|---|---|---|
| *Lepomis gulosus* | 1 | 1 | CC | A |
| *Lepomis gulosus* | 3 | 3 | CC, CC, DM | None |
| *Lepomis gulosus* | 4 | 3 | CC, IM, K | A, B, C |
| *Lepomis gulosus* | 5 | 3 | CC, CC, SB | B, L |
| *Lepomis macrochirus* | 2 | 2 | CC, CC | None |
| *Lepomis macrochirus* | 3 | 2 | CC, IM | B, L |
| *Micropterus salmoides* | 1 | 1 | CC | A, C, L |
| *Micropterus salmoides* | 2 | 2 | CC, IM | None |
| *Micropterus salmoides* | 3 | 2 | IM, SB | B, L |
| *Micropterus salmoides* | 3 | 3 | CC, CC, IM | None |
| *Micropterus salmoides* | 5 | 4 | CC, CC, DM, K | B, C, L |
| *Micropterus salmoides* | 6 | 3 | CC, G, IM | L |
| Total | 38 | 29 | | |

[a]Location: CC = Coelomic cavity, DM = Dorsal musculature, G = gonads, IM = Intestinal mesentery, K = Kidney, SB = Swim bladder

[b]Abnormal Behavior: A = Loss of appetite (refused food), B = Buoyancy problems, C = Convulsions, L = Lethargy

(6.9%); swim bladder, 2 of 29 (6.9%); and ovary, 1 of 29 (3.5%).

Abnormal behavior of was not observed in 4 predators, although larvae (2, 2, 3, and 3 respectively) were collected from these fishes. The mean number of larvae collected from predators with observed aberrant and normal behavior did not differ (Fisher's Exact Test, $P = 0.081$). Aberrant behavior in predators may have depended upon which tissues were damaged and the amount of damage produced by migrating larvae. For example, 2 fish with larvae in the swim bladders exhibited buoyancy problems.

## DISCUSSION

### Distribution

In Florida, the percent of sites with *E. ignotus* in intermediate hosts (fishes) varies regionally, declining from northern to southern Florida. In the present study, 14 of 36 (39%) of sites examined in northern Florida and 11 of 107 (10%) of sites in southern Florida were positive. Frederick et al. (1996) found a similar trend in mosquitofish collected from 69 sites throughout peninsular Florida. They reported that 10 of 24 (42%) of sites in northern Florida and 1 of 45 (2%) of sites in southern Florida were positive. One reason for this difference may be soil type. The parasite was not collected from areas with high organic peat substrate which is typical of soils in southern Florida, while soils in northern Florida are dominated by quartz sand (Brown et al. 1990). In the present study, infected fish were never found at sites with thick peat substrate and positive sites had more silt and clay than negative sites. The natural peat matrix may allow

*Eustrongylides* eggs to settle below the water / peat interphase, thus decreasing the exposure of parasite eggs to potential intermediate hosts.

All positive sites had been altered by human excavation. In southern Florida, sites with natural peat substrate did not have infected fishes and parasite eggs may not be available to intermediate hosts when deposited into the peat matrix. Removal of the peat by excavation exposes the underlying sediment and lime bedrock, where eggs may be more susceptible to ingestion by intermediate hosts. In addition, excavation may reduce the amount of refugia for fish and make them more susceptible to predation by wading birds. Hoffman et al. (1994) reported that wading birds showed a preference for foraging in artificial ponds (dug to improve habitat conditions for deer) in the Everglades. Many of the human-altered positive sites in southern Florida were characterized by clear water, sparse vegetation (primarily emergents and grasses), and large numbers of small fishes (usually livebearers). These fish respond to habitat disturbance with rapid population growth and high densities (Loftus and Eklund 1994). Infected wading birds are probably attracted to these disturbed areas and, if

parasite eggs are deposited, these sites are likely to become foci of infection. In the past, larvae of *Eustrongylides* have been reported in low prevalences from fish collected in southern Florida. From an extensive parasite survey of fishes from southern Florida, Bangham (1939, 1940) recorded single infections of *Eustrongylides* sp. from six species of fishes, including bluegill, bowfin, least killifish, yellow bullhead, spotted gar, and warmouth. During this investigation, he examined 1,380 fishes belonging to 45 species and reported a total prevalence of < 1%. Although the total number of collection sites was not presented, fishes were sampled opportunistically from ditches, lakes and roadside streams throughout southern Florida. It is clear that the parasite has been present in southern Florida for a long period of time, although probably at low prevalences.

Frederick et al. (1996) found an increase in the number of positive northern sites with time. They examined museum specimens of fish that had been collected between 1930 and 1978 from 10 sites and reported that none were infected. Several of these sites are now positive and the parasite may have been introduced recently to northern Florida by

migrating wading birds. Throughout Florida, infected fish were never found at undisturbed natural sites. The increased number of positive sites appears to be related to the association with habitat alteration and urbanization. Alachua Co. (the location of many northern study sites), as well as the entire state of Florida, has undergone rapid human population growth and dramatic changes in land use since the 1930's (Bouvier and Weller 1992).

In this study, sites with a long history (>10 yrs) of human effluent input, were more likely to be the foci of infected fish than any other site type. Of the 21 sewage sites examined, 8 (38%) were positive. In Florida, many aquatic habitats are oligotrophic (Brenner et al. 1990) and long term nutrient input, such as occurs with effluent discharge, creates a gradual eutrophic change in the watershed (Wetzel 1983). In addition, the dissolved oxygen at sewage sites was often low (< 2 mg/l). Fish species were primarily Poeciliidae (mosquitofish and sailfin mollies) which were usually abundant and often observed at or near the surface, but large predatory fish were observed rarely at these sites. Low DO may force the poeciliids, which are able to survive and proliferate in these marginal habitats

(Meffe and Snelson 1989), to the top of the water column where they may be more susceptible to predation by wading birds. Furthermore, larger predatory fish may be unable to survive under such low oxygen conditions.

Sewage effluent and thermal discharge have been associated with high prevalences of *Eustrongylides* in fish, presumably by creating conditions favorable for high densities of intermediate hosts (Hirshfield et al. 1983; Spalding et al. 1993; Frederick et al. 1996). In central Florida, Smith (1992) reported an increase by several orders of magnitude in density and biomass of mosquitofish and least killifish following the input of treated wastewater to forested wetlands when compared to control sites. Poeciliids are important intermediate hosts of *E. ignotus* in Florida and these high densities of fish in wastewater receiving areas may make them especially suitable for outbreaks of eustrongylidosis. Spalding et al. (1993) reported increased densities of oligochaetes in nutrient enriched waters in Florida. In addition, Pezenshki (1987) reported increased abundance of benthic macroinvertebrates, including oligochaetes, when treated wastewater was introduced into existing wetlands in northern Florida.

Ciconiiform bird foraging behavior probably acts to increase the prevalence of the parasite at these sites as well. Wading birds are attracted to sites with high secondary productivity, which is typical of these transitional eutrophic and hypereutrophic areas (Edelson and Collopy 1990; Frederick and McGee 1994). Concentrated wading bird foraging activity (especially if infected birds are involved) would increase the likelihood that parasite eggs would be deposited at the site.

The percent of urban sites that were positive, including retention ponds, canals, and sites adjacent to boat ramps was lower than that at sewage treatment sites (26 vs. 38%, respectively). Many of the same factors associated with increased prevalences of infected fish at sewage treatment sites, such as anthropogenic habitat alteration and nutrient input, were common to urban sites. All of these sites had been excavated and had obvious pollution and nutrient input from stormwater runoff. Fish were usually abundant and easy to catch. Agricultural areas had the lowest percent of positive sites (17%). All positive agricultural sites were excavated and had high secondary productivity also.

Geographic variations in the prevalence of larval *Eustrongylides* in fishes and in the number of aquatic sites with infected fishes have been reported throughout North America. Weisberg et al. (1986) sampled 20 sites in the Chesapeake Bay region (USA) and reported prevalences of larvae of *Eustrongylides* in fish were highest at sites near sewage treatment plants and were negatively correlated with salinity concentrations. Measures (1988*d*) examined fish from 7 lakes in Canada, but reported infected fish from 3 sites only. In both studies, these differences in prevalences were attributed to anthropogenic alterations of aquatic systems, eutrophication, and increased densities of potential intermediate hosts. The number of positive sites may vary both regionally and spatially within a single watershed also. Kaeding (1981) sampled Brown trout (*Salmo trutta*) from one altered site and one unaltered site from the Firehole River in Wyoming (USA). The prevalence of trout infected with larvae of *Eustrongylides* was 44 of 250 (17.6%) at the altered site, while no infected resident fish were collected from the unaltered site.

Fishes

Worldwide, larvae of *Eustrongylides* infect a broad taxonomic range of fish intermediate hosts. Karmanova (1968) reported 14 orders of fishes from which *Eustrongylides* spp. have been collected, including Acipenseriformes, Anguilliformes, Antheriniformes, Beryciformes, Clupeiformes, Cypriniformes, Gadiformes, Gastorosteiformes, Perciformes, Petromyziformes, Pleuroformes, Salmoniformes, Symbranchiformes, and Tetrodontiformes. In Florida, the parasite infects a variety of species of fishes. Bangham (1940) reported six species infected, while Spalding et al. (1993) collected 10 infected species. In the present study, *Eustrongylides* was collected from 11 species. In total, *Eustrongylides* has now been reported from 16 species of fishes in Florida, including *Ameiurus natalis, Amia calva, Fundulus confluentus, F. chrysotus, Gambusia holbrooki, Heterandria formosa, Lepisosteus platyrhincus, Lepomis gulosus, L. macrochirus, L. microlophus, L. puntatus, Lucania goodei, Micropterus salminoides, Poecilia latipina, Pomoxis nigromaculatus, and Xiphophorus variatus.* It is obvious the

parasite is not restricted to fishes of any specific taxonomic group.

Feeding behavior may explain the broad range of infected species. Fishes were both intermediate hosts and paratenic (transport) hosts. When large predatory fish consume infected prey species, larvae of *Eustrongylides* usually survive and remain infective to piscivorous birds (Karmanova 1968). In fact, predatory fish may obtain multiple infections, by consuming several infected prey, and bio-amplify the intensity of parasites. In this study, when infected fish from positive sites in Florida were separated into the broad categories of predator (Centrarchidae and Lepisosteidae) and prey (Cyprinodontidae and Poeciliidae) (Loftus and Kushlan 1987), the prevalence of *Eustrongylides* was higher in predators (4.5%) than prey (1.8%). Based on the percent of parasites collected from infected predatory fish in Florida, Centrarchidae were the most important paratenic hosts, while Poeciliidae were the most important intermediate hosts. Many species of predatory fish may be paratenic hosts, but those that prey on poeciliids are probably most at risk of infection. Of the Poeciliidae, mosquitofish had the highest prevalence of *Eustrongylides*

larvae. Although mosquitofish were the most abundant fish examined from positive sites, this was probably an accurate picture of species composition in the littoral zone of these sites and not the result of sample bias. Mosquitofish are the most abundant and ubiquitous of all fresh water fish species in the southeastern U.S., good colonizers of marginal habitat, and capable of rapid population growth (Loftus and Kushlan 1987; Meffe and Snelson 1989). Of all *Eustrongylides* larvae collected, 89% were found in mosquitofish and they are probably the most important intermediate host fish species in the life cycle of this parasite in Florida.

Infected mosquitofish were collected throughout the year, but the highest prevalence was during winter (December 21 - March 20). This is the dry season in Florida (Chen and Gerber 1990) and, especially in southern Florida, fishes are concentrated in deep water refugia (Loftus and Eklund 1994). Dry season is the period of highest predation by large pisciverous fishes on smaller fish species (Loftus and Kushlan 1987). In fact, Kushlan (1980) concluded that small fish populations may be regulated by predation during these dry periods when fish are forced out of the protective

vegetation and into high densities in more open water. Although there is a time-lag between initial infection of mosquitofish and development of the parasite to the larval stage that is infective to either paratenic hosts (large fish) or definitive hosts (birds), much of the transmission to predatory fish probably takes place during the dry months. These hosts would then become available to wading birds during the spring breeding season.

Infected mosquitofish were smaller (SL and depth) than non-infected mosquitofish. Although female mosquitofish are larger than males generally, there was no difference in prevalence between genders. Age of mosquitofish was not determined and it is possible that younger fish are more susceptible to infection than older fish as a result of differences in habitat use. Juvenile mosquitofish are generally found in close association with vegetation, where they are better able to forage and avoid predation, while adult fish forage in open water more frequently (Belk and Lydeard 1994). Although mosquitofish may have become infected as juveniles, fish with advanced-stage larvae were mature sexually. Alternatively, larvae of *Eustrongylides*

were large (up to 11 cm) and inhibition of growth in small fish may result from the physiological cost of parasitism.

Mosquitofish are diurnal omnivorous surface feeders and the diet consists mainly of insect larvae, zooplankton, larval fishes, and small amounts of vegetation (Meffe and Snelson 1989; Linden and Chech 1990; Belk and Lydeard 1994). The eyes and mouth parts are oriented upward and they are attracted to disturbances on the water surface (Kushlan 1973). During field collections, mosquitofish were usually at or near the surface. I found no published record of mosquitofish predation on oligochaetes, although this is probable, especially in shallow areas of the littoral zone where the surface and sediment are closely associated. Oligochaetes were not identified in stomach contents of mosquitofish from positive sites, but oligochaetes are soft-bodied and probably would be broken down rapidly in fish (Measures 1989d), and therefore may not have been detected.

Oligochaetes

Oligochaetes were more abundant at *Eustrongylides* positive than negative sites. The importance of oligochaete densities as an indicator of aquatic environmental quality is well documented and shown to be positively correlated

with increasing nutrient input (Brinkhurst 1986, Lazim and Learner 1987, and Simpson et al. 1993). Although some researchers believe that sediment physico-chemical composition is the major determinant of oligochaete distribution and density, Brinkhurst (1967) found that neither particle size nor organic content of the substratum could satisfactorily explain the distribution and abundance of tubificid species in his study. He suggested that the type of organic matter and bacterial flora present in the sediment might be important. McMurtry et al. (1983) concluded that microbial constituents in the sediment were more important than physico-chemical properties in determining tubificid densities. Wetzel (1983) reported increased bacteria density at sites with anthropogenic nutrient input which resulted in increased oligochaete densities. In the present study, the higher density of oligochaetes at positive sites was probably the result of anthropogenic habitat alterations such as sediment disturbance and nutrient input which increased benthic productivity.

Higher densities of oligochaetes were found at positive and negative study sites during the wet season (March -

October) than during the dry season (November - April). In temperate and sub-tropical latitudes, many species of oligochaetes were reported to have annual reproductive cycles with population peaks during late spring, summer, and early fall (Block et al. 1982). Therefore, in Florida, increased densities of oligochaetes during the wet season were probably the result of natural population cycling.

Aquatic oligochaetes were identified as the first intermediate host of *E. excisus* by Karmanova (1968) in the Volga River delta (Russia). She reported three species of oligochaetes (*Lumbricus variegatus, Tubifex tubifex*, and *Limnodrilus* sp.) were infected with *Eustrongylides* under experimental conditions. In her studies, several experimentally infected oligochaetes were sexually immature. Although development of the parasite to the third larval stage was described in oligochaetes, it was unclear if *Eustrongylides* was experimentally transmitted from oligochaetes to fish. To date, no naturally infected oligochaetes have been reported from the eastern hemisphere.

In North America, Measures (1988*c*) implicated two species of tubificids *(Limnodrilus hoffmeisteri* and *Tubifex tubifex*) as intermediate hosts of *E. tubifex*. Both species

of oligochaetes were common in areas with infected fish, although many specimens were sexually immature and no naturally infected oligochaetes were collected. Lichtenfels and Stroup (1985) reported 1 third-stage larval *Eustrongylides* sp. from an immature tubificid oligochaete from the Chesapeake Bay area (USA). As the taxonomic classification of both nematodes and oligochaetes is based on adult characteristics, identification to species was not possible. However, of the oligochaetes collected ($n$ = 1,767), all of the mature tubificids were identified as either *Limnodrilus hoffmeisteri* or *L. cervix* and it was proposed that the infected oligochaete was probably a *Limnodrilus* sp. In the present study and Spalding et al. 1993, immature tubificids and *L. hoffmeisteri* were the most common oligochaetes identified from enzootic sites, although no naturally infected oligochaetes were observed.

At least four reasons may account for the low prevalence of parasite larvae in naturally infected oligochaetes: First, the parasite probably develops in a small percentage of naturally exposed oligochaetes. Second, infected oligochaetes may be over dispersed and found only at very small foci in the littoral zone. This may be the

result of parasite eggs being concentrated in small areas when birds defecate and the detection of these areas are difficult. Third, epizootics of eustrongylidosis in oligochaetes, fish, and birds are probably dynamic over time. At the time larvae of *Eustrongylides* are first detected in fish, many infected oligochaetes may have either been consumed by predators or perished naturally. Unless parasite eggs are distributed in the area routinely, the prevalence of infected oligochaetes would decline through natural mortality.

Physico-chemical Characteristics of Water and Sediment

Many researchers have suggested a link between eustrongylidosis and anthropogenic alterations of aquatic systems, especially nutrient input (Hirshfield et al. 1983; Measures 1*988c*; Spalding et al. 1993). In the present study, all sites with infected fishes had obvious anthropogenic nutrient input. Many of these sites have undergone eutrophication within the past 50 years. Brenner et al. (1990) reviewed available chemical data from aquatic systems in Florida and showed that trophic states ranged from ultra-oligotrophic in the sandhills to hyper-eutrophic near muck farms, sewage plants, and phosphate deposits.

Although it may be tempting to assume that subtropical aquatic systems with karst substrate may be naturally eutrophic, this is not true in Florida. In fact, the majority of Florida lakes were classified as oligotrophic or mesotrophic by Canfield et al. (1983). Many of Florida's lakes and fresh water marshes have soft clear water which is naturally acidic and deficient in nitrogen and phosphorus. These conditions make them vulnerable to dramatic change in trophic state, brought about by the addition of water-borne nutrients, such as has occurred in Lake Apopka (Brenner et al. 1990). Although not understood completely, there appears to be a cascade effect of eutrophication in which some aquatic species become scarce or disappear and others become dominant. An example would be the decline of largemouth bass and increase of gizzard shad in Lake Apopka. In addition, the historically clear waters of Lake Apopka are now subject to frequent and persistent algal blooms.

Water at positive sites was characterized by lower dissolved oxygen (DO) than at negative sites. These oxygen concentrations were often < 2 mg/l, which is characteristic of hyper-eutrophic waters (Wetzel 1983). One exception to this observation was at positive sites with large algae

blooms, such as Lake Apopka and some retention ponds. At these sites, DO often increased during daylight hours because of algal photosynthesis, but declined to nearly undetectable limits during the night, when algae reverted to respiration and became oxygen sinks.

Belanger et al. (1989) reported from the Everglades that periphyton algae contribute the most DO to the system, while emergent plants created an oxygen sink when dead plant material decomposed under water. Browder et al. (1994) showed that DO was significantly higher at a low nutrient Everglades site than at a nutrient-enriched site, where native periphyton communities were negatively impacted. At the low nutrient site, DO ranged from 1.3 - 8.2 mg/l, while values did not exceed 1.2 mg/l at the nutrient-enriched site. Subtle changes in vegetation and algal communities, as a result of nutrient enrichment, have had a negative impact on habitat quality in the Everglades and have been accompanied with reduction of DO in many areas.

This pattern of oxygen depletion has been repeated in other aquatic systems, such as during the rapid anthropogenic eutrophication of Lake Erie (Carr 1962) and was usually accompanied by dramatic changes in aquatic and

benthic fauna. Some species of oligochaetes, such as *Limnodrilus*, thrive in low oxygen environments and may become the dominant benthic invertebrate (Kennedy 1965, 1966). Low DO may eliminate less tolerant species of benthic invertebrates or aquatic macrofauna, such as fish, and reduce predation on surviving oligochaetes.

Fish kills, brought on by low DO, are common in Florida and involve large-bodied fishes primarily (Loftus and Kushlan 1987). Small-bodied fish species, such as cyprinodontids and poeciliids appear to survive these events and may even become more abundant when predation pressure is reduced. Poeciliids especially, are good colonizers of marginal habitat and able to survive by remaining near the surface and taking advantage of atmospheric oxygen when DO is reduced (Meffe and Snelson 1989). In addition, by forcing fish to the top of the water column for respiration, low DO may increase the risk of predation on these fish by wading birds.

In the sediment, soil oxygen demand (SOD) was higher at positive than negative sites. Soil oxygen demand in some systems, such as the shallow productive sites in this study, may increase the depletion of DO in the water column (Gale

et al. 1992). Increased SOD is the result of microbial action and respiration of benthic invertebrates (Wetzel 1983). High SOD at positive sites probably resulted from bacterial decomposition of organic detritus. These anoxic conditions favor ubiquitous oligochaete species, such as *L. hoffmeisteri*, which further increase oxygen demand. Moore (1981) reported that the main food source of aquatic oligochaetes was bacteria, but that large amounts of bottom sediment such as dead algae were consumed in the process of extracting the bacteria. In fact, dead algae account for 57 - 75% by volume of ingested matter in some species of oligochaetes (Moore 1981). Under natural conditions, eggs of *E. ignotus* are probably ingested by oligochaetes when consuming this detritus.

Total carbon (TC) in the water columns of positive sites was higher than negative sites during the wet season 1996. Most of the difference appeared to be in the inorganic fraction. The carbon cycle in fresh water systems occurs from the balance of $CO_2$ fixed to form organic compounds and the oxidation of organic carbon back to $CO_2$, mediated by phytoplankton and microbial action. In most mesotrophic aquatic systems, as $CO_2$ dissolves in water, the

solution contains the same approximate concentration by volume as in the atmosphere (Broecker et al. 1979). In eutrophic systems, fixation of $CO_2$ by photosynthetic algae is the most important source of carbon input and as phytoplankton biomass increases, a similar increase is observed in total carbon (Gale and Reddy 1994).

Anthropogenic sources of carbon may include nonpoint (precipitation and stormwater runoff) or point source (drainage from streams and canals with wastewater treatment or industrial discharge). In a carbon flux model of Lake Apopka (hypereutrophic system), Gale and Reddy (1994) reported that fixation of $CO_2$ by phytoplankton was the major contributor to the annual carbon budget, although anthropogenic point source input of carbon was significant also.

Although photosynthesis and respiration are the two major factors influencing the amount of carbon in the water column, anoxic decomposition in the sediment is a factor also (Kerr 1973). The undecomposed carbon leaves the system through outflow or is bound in the sediment. Many of the positive sites were characterized by anoxic conditions and

disturbed sediments, which could expose more carbon to microbial action and re-suspension into the water column.

Carbon alone probably plays a minor role in the epizootiology of *E. ignotus* in most systems. Microbial detritivores, which transform organic carbon to the inorganic state, help to create anoxic conditions in the benthic substrate and provide food for oligochaetes. As discussed earlier, these anaerobic environments probably have dramatic effects on potential intermediate hosts by excluding some species of oligochaetes and fish and favoring others.

Nitrogen and phosphorus have long been recognized as the major macronutrients which limit the productivity of aquatic systems and concentrations are used to estimate the the trophic status of watersheds (Huber et al. 1982). The mean TN and TP in surface water were higher at positive sites during all seasons, which probably indicates higher trophic state, than negative sites. In Florida, land use patterns influence the amount of nitrogen and phosphorus input from exogenous sources such as agriculture, mining, and urban and wastewater runoff (Brenner et al. 1992). In the Everglades, productivity is reported to be limited by

phosphorus input (Davis 1994), but Canfield (1983) proposed that some central Florida lakes received excessive amounts of phosphorus from natural sources or phosphate mines and were nitrogen limited. Although anthropogenic activity is a major source of exogenous nitrogen, phosphorus concentrations in Florida surface waters have great variance, due to geological formations such as phosphate deposits in drainage basins (Nordlie 1992). Increased nitrogen and phosphorus input alone does not cause outbreaks of *Eustrongylides* in intermediate hosts, but the cascade effect of increased productivity, as a result of these inputs, may result in increased densities of potential intermediate hosts and make these sites attractive to foraging wading birds.

Huber et al. (1982) reported that aquatic systems with N / P below 10 were nitrogen limited and those with N / P above 20 were phosphorus limited. Nitrogen / phosphorus ratios were lower at positive than negative sites and positive sites appeared to be nitrogen limited, while negative sites were phosphorus limited. Nitrogen nutrient limitation is often an indication of eutrophication of aquatic systems (Wetzel 1983).

Chlorophyll-a concentrations were higher at positive than negative sites also. Canfield et al. (1983) suggested that chlorophyll-a was an accurate measure of algae concentrations in the water column, but that system productivity in Florida lakes should not be inferred because macrophyte productivity is not included. Most researchers report that increased chlorophyll-a concentration is a reliable indication of increased primary productivity (Brenner et al. 1992).

Mean sediment grain size was greater at positive than negative sites. Sediment particle size is a result of erosion and sedimentation processes in the watershed (Waters 1995). Natural erosion, in the absence of human activity, is affected by rainfall, water flow, vegetation and basin morphology in the watershed. On the other hand, anthropogenic factors include disturbance in the drainage basin by urban development, agriculture, mining, and forestry. In a land-use study from the southeastern U. S., Jones and Holmes (1985) compared urban, agricultural, and forested areas, summarizing the effects of land-use on state water resources. They concluded that urban development contributed the most sediment and other pollutants to

watersheds, followed by agriculture and mining, while forestry practices contributed little. Frequent anthropogenic disturbance to aquatic sediment (dredging) and habitat disturbance in the surrounding drainage basin have a profound effect on sediment particle size distribution, by increasing erosion and the amount of silt deposited in the system.

In the present study, positive sites had more sediment heterogeneity (higher percent silt and clay) than negative sites. Positive sites had greater densities of oligochaetes also. The dependence of benthic invertebrates on substrate particle size and heterogeneity was observed in other studies (Culp et al. 1983, Waters 1995). Although the abundance of benthos correlated positively with particle size, Minshall (1984) pointed out that the more functional relationship may be between benthos abundance and substrate heterogeneity, including the amount of organic detritus. Without organic detritus as a food source, Culp et al. (1983) reported that density and biomass of benthic invertebrates decreased significantly regardless of substrate particle size. Benthos abundance was least in

homogeneous sand, silt, or clay and greatest in heterogeneous mixtures.

Fish abundance and community structure is often associated with sediment particle size because of availability of benthic organisms as a food resource and preference of sediment types as substrate for nest building (Waters 1995). Of the principal taxa available to fish, insects such as Ephemeroptera, Plecoptera, and Trichoptera require a heterogeneous mixture of large particles, while higher densities of burrowing organisms such as chironomids and oligochaetes often occur in a mixture of silt and clay (Culp et al. 1984). It appears that excavation and the resulting impacts of erosion may be important in the life cycle of *E. ignotus*, as positive sites often had a history of frequent disturbance, while natural undisturbed sites were always negative.

Vegetation

Aquatic macrophytes create essential habitat for high densities of small fish (Loftus and Eklund 1994). In the Everglades, Loftus and Kushlan (1987) found that vegetation inhibited the movement and feeding of large fishes, thereby acting as refuges for small killifishes, livebearers, and

juvenile sunfishes. Densities of small fishes declined when water levels decreased and fishes were forced out of aquatic vegetation and into deeper water, where predation was thought to increase. Aquatic vegetation in the littoral zones of lakes provided similar refuges and small fishes were at increased risk of predation when water levels declined in these habitats also (Werner and Hall 1977). It appears that high densities of small fish intermediate hosts are essential for the life cycle of *E. ignotus* and these densities may be dependent upon aquatic macrophytes for refuge from larger fish predators.

Positive sites were characterized by higher percent grasses and emergent vegetation than negative sites. Hoffman et al. (1994) reported that open grass and emergent vegetation communities were more attractive to foraging wading birds than areas with dense aquatic vegetation. Although larger birds were able to forage in deeper water, Powell (1987) found many foraging wading birds concentrated in shallow areas which were characterized by grasses, emergent vegetation, and high densities of prey items. Grasses and emergent vegetation may restrict the movement of larger fish predators but provide little protection from

vertical attack by birds. On the other hand, areas with floating vegetation are characteristic of deeper water with more stable hydroperiod (Wetzel 1983), and are less attractive to wading birds.

In Florida, most grasses and emergents are either annuals or perennials and provide a continuous source of detritus to the benthos (Tarver et al. 1988). Although living macrophytes are generally not an available macroinvertebrate food resource, decaying plant material and the bacteria associated with this detritus can be an important energy source for oligochaetes (Wetzel 1983). In combination with the accumulation of dead algae, deposition of organic material from the periodic cycling of grasses and emergents provide good habitat and food resources for oligochaetes.

Grasses and emergents are often the first macrophyte colonizers of disturbed aquatic habitats, while floating and submerged vegetation are characteristic of stable systems with long hydroperiods (Toy and Hadley 1987). In Florida, these aquatic plant associations vary over time and major disturbances are defined by fire frequency, changes in nutrient input, hydrologic regime, and soil (Kushlan 1992).

With the exception of fire, most positive sites were characterized by frequent anthropogenic disturbances such as nutrient input from sewage effluent or runoff, hydrologic fluctuations in canals and retention ponds due to rainfall events, and excavation of these watersheds to remove vegetation and accumulated sediment. The dominant vegetation communities at positive sites are the result of these disturbances.

Lake Alice

A large wading bird breeding colony was observed on Lake Alice for many years prior to the present study (Karraker 1953). By the 1980's, this colony had been abandoned and wading birds were only observed roosting in the watershed (Korhnak 1996). Several factors may have led to the abandonment of this colony, including changes in hydrology, loss of suitible habitat, or disease. Many of the trees and woody shrubs used by nesting birds are no longer present. Fish infected with eustrongylid larvae were first identified from the Lake Alice sewage treatment plant outflow in 1990 by Spalding et al. (1993). In 1990 and 1991, 90 (38%) of 234 fish collected from the site were infected. Although not investigated, infected fishes were

probably in the system prior to 1990 and may have been a factor in the abandonment of the wading bird colony.

The advanced wastewater treatment plant was completed in October 1994 and began operation on November 18, 1994. Most treated effluent was piped directly to the injection wells, with additional water used for irrigation of a municipal golf course (Korhnak 1996). Poeciliids (especially mosquitofish) were the most common infected fish collected at the treatment plant outflow and prevalence was 13 (33%) of 40 in November 1994. Prevalences of infected fish declined throughout the watershed in 1995 and 1996 and no infected fish were collected in 1997. Sites with infected fishes appeared to be very localized and associated with the sewage treatment plant outflow. Other researchers reported similar findings and infected fish were often restricted to sites that received anthropogenic nutrient input (Hirshfield et al. 1983; Spalding et al. 1993).

Poeciliids probably became infected and remained near the same area for the duration of their lives. Because poeciliids are small and difficult to mark or tag by conventional methods, little is known of their movement patterns (Meffe and Snelson 1989). In the absence of

stochastic events such as flooding or drought, mosquitofish were thought to remain near their natal site (Meffe and Snelson 1989). Meffe (1984) studied effects of flash flooding on community structure of poeciliids in Arizona and determined that native poeciliids were able to survive flooding and remained at pre-flood sites. Although sites in the present study were subject to frequent flooding due to rainfall events, infected fishes appeared to find refuge in vegetation or near human-made structures.

Discharge from the wastewater treatment plant was a major source of nutrient input to the watershed for many years (Mitsch 1975, Vega and Ewel 1981, Korhnak 1996). Korhnak (1996) estimated that effluent from the wastewater treatment plant contributed 65% of TP and 78% of TN loading to the system. In the 7-months following diversion of wastewater, Korhnak (1996) reported declines in mean in-lake TP concentrations from 1.14 mg/L to 0.59 mg/L and from 2.50 mg/m3 to 0.93 mg/L for TN. In the present study, similar declines in TP and TN were observed. Clearly, diversion of effluent led to a dramatic decline in phosphorus and nitrogen loading to the Lake Alice system. This decline was

accompanied by a decline in the prevalence of infected fish also.

There was no significant change in the amount of inorganic or organic carbon for 3-yrs following effluent removal. Although phosphorus and nitrogen may limit primary productivity more frequently, inorganic carbon is a major nutrient of photosynthetic metabolism (Wetzel 1983). In aquatic systems, the major source of inorganic carbon is atmospheric CO2, while most organic carbon (both dissolved and particulate) occurs in dead organic matter. Atmospheric inputs probably did not change significantly over the course of the study and thus the lack of change in inorganic carbon values was not surprising.

The Lake Alice watershed was a highly productive system for many years and densely populated by floating and emergent macrophytes from the late 1960's (Vega 1978). An algal bloom, throughout the system, was observed in spring 1995 following 3-weeks of warm dry weather. Algae may play an important role in the life cycle of *E. ignotus*, by providing a food resource for fish and a detrital substrate for oligochaetes and other aquatic invertebrates.

The upstream site (A) probably received minimal nutrient input from the wastewater treatment plant. Throughout the study, there was intensive development in the upper watershed, including construction of buildings and parking lots, which may have increased runoff from impervious surfaces. This development was probably the most important factor in the decline of Chlorophyll-a in the upstream watershed, as increased runoff often results in increased turbidity and reduction of Chlorophyll-a in streams (Waters 1995). In many aquatic systems, chlorophyll-a concentration was shown to be correlated with nitrogen and phosphorus loading (Mason 1991) and diversion of effluent was probably a major factor in the reduction of algal Chlorophyll-a from the input study site (B) and downstream throughout the remainder of the system.

Although it is tempting to infer a cause and effect relationship between the decline of infected fish in the watershed and termination of wastewater discharge, this is not clear. Potential intermediate hosts of *E. ignotus* were present throughout the study. Although I observed wading birds foraging in the area throughout the study, infected birds may have died or migrated to other areas and parasite

eggs may not have been deposited at the sites. Natural mortality of infected fish, with no source of recruitment, could explain the decline in numbers of infected fish.

## Life Cycle

### Eggs

Under experimental conditions, eggs of *E. ignotus* were infective to intermediate hosts after 17 to 21-days incubation. Development of eggs was temperature-dependent, with more rapid development associated with higher incubation temperatures. Under natural conditions, the time required for development of mature eggs in the environment may be less. Experimental eggs were obtained from gravid worms, thus reducing the amount of time eggs would incubate inside the definitive host before passing into the environment with feces. This may have resulted in increased amount of time required for maturation.

Adult female *E. ignotus* produced and oviposited eggs with shells, regardless of whether fertilization had taken place. This was similar to observations of eggs of *E. tubifex* by Measures (1988a). Fertilized eggs had a thick pitted shell. Although mamilalation and pitting of the egg shell is characteristic of all Dioctophymids, the shells

*Eustrongylides* appears to be the most irregular and have the highest number of pits (Karmanova 1968). The structure of the shell may be important in the life cycle of the parasite, as irregularly shaped pits provide points of attachment which allow the eggs to adhere to living vegetation and detritus. Eggs, which attach to potential food resources of intermediate hosts, are probably more likely to be ingested. In addition, eggs which adhere to rooted vegetation are probably less likely to be transported away from the wading bird foraging site by water currents. Under natural conditions, eggs probably adhere to vegetation throughout the water column. Bacteria were often observed in egg shell pits also. Many tubificid oligochaetes feed on the bacteria associated with detritus (Brinkhurst 1967, Brinkhurst and Chua 1969) and the presence of bacteria on the egg shell may increase the probability that the egg will be consumed by an oligochaete.

Eggs of *E. ignotus* were able to survive wide temperature variations and development of larvae appeared to be temperature dependent. This was reported also for *E. tubifex* (Measures 1988), *E. excisus* (Karmanova 1968), and *Dioctophyma renale* (Mace and Anderson 1975). Measures (1988) observed that eggs of *E. tubifex* did not develop at 4 C but

remained viable for 2.5 yrs, suggesting an adaptation for overwintering in northern latitudes. In the present study, *E. ignotus* eggs developed more rapidly in warm water with temperatures typical of summer in Florida. Under natural conditions, rapid development of eggs in warm water probably increases the likelihood of transmission to intermediate hosts because poeciliid fishes and oligochaetes are more abundant during that time.

Under experimental conditions, eggs did not develop in hypersaline solutions (seawater). This was probably a result of osmotic changes, as many eggs became crenated or ruptured. Although outbreaks of eustrongylidosis have been reported from estuarine environments, such as the Chesepeake Bay area (USA), most outbreaks were confined to freshwater tributaries or discharge canals (Hirshfield et al. 1983). In Florida, *E. ignotus* appears to be restricted to freshwater habitats (Spalding et al. 1993) and infections have never been reported from marine fishes. In addition, no *Limnodrilus* spp. are known to to survive in marine habitats (Kennedy 1965).

The parasite life cycle is vulnerable to interruption by drydown events at several points. Eggs of *E. ignotus*

required an aquatic media for development and were susceptible to dehydration. Under experimental drydown conditions, few eggs developed to the first larval stage, although interstitial moisture in the sediment may provide some protection if the drydown is not complete. Lukasiak (1930) recorded similar findings in his work with *D. renale* when he reported: "Eggs perished when mixed with soil, dried in the sun, and re-soaked with water". In Florida, drydown events of ≥ 60-days would probably eliminate most infective eggs from the environment. In addition, potential intermediate hosts (fish and oligochaetes) would be removed.

*Eustrongylides ignotus* in Oligochaetes

In North America, at least 2 species of tubificids (*L. hoffmeisteri* and *T. tubifex*) have been implicated as intermediate hosts of *Eustrongylides* spp. Measures (1988c) infected both of these species with *E. tubifex* experimentally, while in the present study *L. hoffmeisteri* was infected with *E. ignotus*. Lichtenfels and Stroup (1985) identified 1 naturally infected aquatic oligochaete as an immature Tubificidae resembling *Limnodrilus*. Although both *L. hoffmeisteri* and *T. tubifex* are distributed widely

throughout North America, only *L. hoffmeisteri* was identified from wading bird foraging areas in Florida.

In the present study, mature *L. hoffmeisteri* and immature *Limnodrilus* sp. were infected in the laboratory. Presumptive identification of immature *L. hoffmeisteri* is often based on the abundance of adults in the sample because *Limnodrilus* may lose and regenerate reproductive organs several times over the life of the organism (Brinkhurst and Kennedy 1965). As all infected adults were identified as *L. hoffmeisteri*, immatures were probably offspring of *L. hoffmeisteri* that had reproduced asexually or adults that had lost reproductive organs through senescence.

Whether infection with *E. ignotus* caused the loss of reproductive organs in the oligochaetes or not was unclear. Although larvae first developed in the ventral blood vessel, several advanced-stage larvae appeared to be free in the coelom. The mouth of the parasite larva was normally observed in the segments of the oligochaete containing the gonads. These are the most highly vascularized segments and presumably provide optimal nutrition for development of the parasite. The nutritional demands of the parasite or other factors, such as physical or chemical taxis may initiate re-

absorption of the reproductive organs or inhibit sexual maturation of oligochaetes.

*Limnodrilus hoffmeisteri* is the most important oligochaete intermediate host of *E. ignotus* in Florida and provides an indirect mode of transmission to fish. *Limnodrilus hoffmeisteri* is cosmopolitan in distribution and may dominate oligochaete species assemblages in organically enriched or grossly polluted waters (Stimpson et al. 1982; Schloesser et al. 1995). *Limnodrilus hoffmeisteri* survive for up to 3 years (Poddubnaya 1980) and can maintain the infective larva for long periods of time in the environment. Transmission to fish through oligochaetes is probably an adaptation for maintaining the parasite in the environment when eggs are not consumed by fish directly.

Multiple infections of oligochaetes were observed infrequently. Larvae, which were large in body size, probably caused mortality in oligochaetes with multiple infections. Alternatively, larvae may compete for optimum sites in the oligochaetes and few larvae survive. Measures (1988a) reported similar findings in *E.* tubifex. When multiple infections of *E. tubifex* were observed, the larvae

were significantly smaller than those in oligochaetes with single infections.

Parasite development in oligochaetes was temperature dependent, with greater numbers of infected oligochaetes and more rapid development at 28 C than at 19 C. Measures (1988*c*) suggested that temperature dependent development was an adaptation to population fluxes in intermediate and definitive hosts. This allowed *E. tubifex* to be transmitted to migratory birds during the limited time when birds were present in northern latitudes. In Florida, birds are present during all seasons and parasite eggs are deposited throughout the year. Intermediate hosts were more abundant in the warm (wet) season and this is when most infection of fish probably takes place, although not detected until winter.

In the laboratory, oligochaetes ingested eggs which hatched or passed through the annelid's digestive system within 48-hrs. Although no first-stage larvae were detected in oligochaetes, they probably were overlooked. Measures (1988*a*) was unable to detect first-stage larvae of *E. tubifex* in oligochaetes also and attributed this to their small size and relative immobility. Karmanova (1985)

reported that eggs of *E. excisus* hatched in the gut of the oligochaete, where they developed and migrated into the ventral blood vessel.

The cephalic region of all L-1 *Eustrongylides* are characterized by a stylet. Karmanova (1968) suggested that larvae may secrete a lytic compound and utilize the stylet to pierce host tissue and migrate into the abdominal blood vessel of the oligochaete. The larva then localized in the blood vessel, where the next molt took place and the stylet was lost.

In the present study, second-stage larvae were first observed in the ventral blood vessel of *L. hoffmeisteri* at 28-days PI and retained the cuticle of the first molt. Apparently, humoral components of the oligochaete were utilized for nutrition. It was unclear why larvae located in the abdominal and not the in the dorsal blood vessel. In oligochaetes, blood is transported anteriorly in the dorsal vessel to the abdominal blood vessel (Brinkhurst and Jamieson 1971). Blood flow is maintained by a series of pseudo-hearts, one-way valves, and peristalsis of the dorsal vessel. Measures (1988*c*) suggested that the dorsal blood vessel may not be suitable for development of larvae because

of these one-way valves, which could restrict mobility and room for growth. Larvae in the dorsal vessel could become trapped and occlude the vessel, killing the host. In addition, the large abdominal vessel may be easier for larvae to locate and penetrate.

Larvae of *E. ignotus* developed more rapidly in oligochaetes when compared with those of *E. excisus* or *E. tubifex*. Third-stage larvae of *E. ignotus* were first observed in oligochaetes at 35-days PI, while L-3 *E. excisus* was first detected at 80-days PI, and L-3 *E. tubifex* were observed at 70-days PI (Karmanova 1968, Measures 1988*c*). Although third-stage larvae of *E. ignotus* were first observed at 35-days PI, the larvae appeared to require ≥ 180-days in the oligochaete before they were infective to fish. Third-stage larvae of *E. ignotus* survived in oligochaetes for long periods of time (≥ 284-days) and may serve as a source of infection to fish for the life of the oligochaete.

Although thousands of oligochaetes from enzootic areas were examined, no naturally infected oligochaetes were observed. Under natural conditions, infected oligochaetes probably occur in patchy distributions. Several factors may

influence the distribution of infected oligochaetes, such as the number of infected birds foraging in the area, the frequency of visits, and the number of parasite eggs that fall to the sediment and mature. In addition, eggs probably fall to the sediment in a small area, near the point of defecation. Tubificid oligochaetes are relatively immobile (Brinkhurst 1986) and parasite eggs must settle near the burrow to be available for ingestion.

In North America, 1 naturally infected oligochaete was reported from the Chesapeake Bay area by Lichtenfels and Stroup (1985). The authors did not indicate the number of cuticles retained by the larva, but morphology and length were similar to late L-2 or early L-3 *E. ignotus* larva from experimentally infected oligochaetes in the present study. In addition, only adult *E. ignotus* have been reported from bird definitive hosts in the Chesapeake Bay area (Lichtenfels and Pilitt 1986).

*Eustrongylides ignotus* in Fishes

In North America, experimental transmission of larval dioctophymids to fish intermediate hosts has proven difficult. Mace and Anderson (1975) fed oligochaetes (*Lumbriculus variegatus*) infected with L-3 *D. renale* to 15

channel catfish (*Ictalurus punctatus*) and 15 brown bullheads (*I. nebulosus*). Larvae appeared to be digested by the fish and none was infected 30-days PI. Measures (1988*d*) fed infected oligochaetes (90 - 109-days PI) to five pumpkinseeds (*Lepomis gibosus*) and none was infected 13 - 34-days PI. She suggested that larvae may require further development in oligochaetes before they were infective to fish. In the present study, 94 mosquitofish, 8 warmouth, and 3 largemouth bass were each fed oligochaetes infected with second and third-stage larvae of *E. ignotus*. Thirty-one mosquitofish, 2 warmouth, and 1 largemouth bass were each fed oligochaetes with second-stage larvae (< 35 days-PI) and did not become infected. The remaining 71 fishes were fed oligochaetes with L-3 (< 180 days-PI) and 2 mosquitofish were infected. One mosquitofish, examined at 127-days PI was infected with an encapsulated third-stage larva, and another mosquitofish, examined at 184-days PI, was infected with an encapsulated fourth-stage larva. Although *E. ignotus* was transmitted to fish from infected oligochaetes, prevalences were less than expected. Second-stage larvae were not infective to fish, although this may have been due to low sample size of second-stage larvae used

in experimental transmission work. It appears that larvae must develop in oligochaetes for ≥ 127 days before they are infective to fish. Under natural conditions, fish probably consume many infected oligochaetes before they become infected in this manner.

Based on natural prevalence and experimental studies, poeciliid fishes (especially mosquitofish) were the most important intermediate host of *E. ignotus*. Mosquitofish are the most abundant and ubiquitous fish in Florida fresh water systems and are excellent colonizers of marginal habitat (Loftus and Kushlan 1987). Mosquitofish are omnivorous, visual-foraging surface feeders, attracted to surface disturbance such as the movement of insects or other potential prey items. The mouths of mosquitofish are dorso-terminal and the eyes are oriented toward the surface. In the present study, mosquitofish consumed oligochaetes that were placed on the surface or allowed to descend through the water column of experimental tanks. However, if worms fell to the sediment and formed burrows, they were not captured. If oligochaetes are encountered by mosquitofish naturally, it is probably in very shallow water or near shore, where the distance between surface and sediment is minimal.

For several reasons, including the tendency of parasite eggs to adhere to vegetation throughout the water column, the morphology and feeding behavior of mosquitofish, and the difficulty of transmission to fish from infected oligochaetes, mature eggs were fed directly to mosquitofish. In a pilot study, 2 of 105 (1.9%) mosquitofish became infected after ingesting eggs. In later experiments, 11 of 194 (5.7%) became infected after ingesting eggs. Although no first-stage larvae were observed, they are inconspicuous and undoubtably were overlooked during examination. Eggs probably hatch in the stomach or intestine shortly after ingestion, migrate out of the gut, and mature to the second-stage in vascularized tissue or blood vessels near the gonads. Third-stage larvae become encysted in the reproductive tract or attach to the mesentery of the coelomic cavity and they mature to the fourth-stage by 105-days PI. At this time, larvae are infective to paratenic hosts (predatory fishes) or to definitive hosts (birds).

It was unclear why single infections only were observed in experimental fish fed several eggs. Multiple infections were observed rarely in naturally infected mosquitofish, although another researcher (M.G. Spalding pers. comm.)

observed multiple infections in mosquitofish collected from the Lake Alice watershed in 1990. Measures (1988*d*) reported that single infections of *E. tubifex* were more common than multiple infections in fish. The body size of *E. ignotus* larvae are large in comparison with that of mosquitofish and immunologic response of the host may inhibit development of multiple infections, increasing the possibility that only one larva will mature to the infective stage. More likely, there may be competitive inhibition of larvae for optimal infection sites within the intermediate host.

This is the first report of development of a dioctophymid larva in a fish without an annelid intermediate host. It is not known if this life cycle is unique to *E. ignotus* or if other *Eustrongylides* spp. develop directly in fish as well. Climate may be a factor in this adaptation. *Eustrongylides ignotus* is distributed throughout temperate and tropical habitats, while *E. tubifex* and *E. excisus* appear to be restricted to northern latitudes primarily and birds become infected in late summer (Measures 1988*a*). In northern latitudes, oligochaetes are probably a more efficient intermediate host of *Eustrongylides*, due to freeze-up and limited time available for transmission to

migrating of birds. In Florida, wading birds were infected and produced parasite eggs throughout the year (Spalding et al. 1993). Infected fish were collected during all seasons also and it is not necessary for larvae to over-winter before transmission to the definitive host. In Florida, direct transmission to fish may be the most efficient and most important life cycle strategy.

Large numbers of parasite eggs in the environment are probably necessary for completion of the life cycle under natural conditions, regardless of transmission mode. Experimentally, fish were difficult to infect and repeated ingestion of infected oligochaetes or eggs may be required to produce high prevalences in fish. Infected birds are probably attracted to small eutrophic sites, where high densities of fish and parasite eggs are concentrated. These sites have high densities of oligochaetes also. When large numbers of fish, oligochaetes, and parasite eggs are present in the environment, transmission to foraging wading birds is likely.

*Eustrongylides ignotus* in Paratenic and Abnormal Hosts

The life cycle of *Eustrongylides* is the most complex of any nematode in the order Diocyophymidea (Karmanova 1968). Development of *Eustrongylides* spp. can take place in three hosts (trixenous), although *E. ignotus* was discovered to be both trixenous and dixenous in this study. Karmanova (1968) suggested that the complex life cycle of *Eustrongylides* probably developed recently and may help account for the cosmopolitan distribution of this genus, as parasites with a broad range of intermediate hosts are widely distributed.

In addition to intermediate hosts, researchers reported that paratenic hosts may be important in the distribution of some species of dioctophymids. Mace and Anderson (1975) observed natural paratenic infections of *D. renale* in fish and frogs. Under experimental conditions, the authors transferred the parasite from frogs (*Rana clamitans melanota* and *R. pipiens*) to the definitive host, mink (*Mustela vison*). *Eustrongylides* spp. have been reported from a variety of paratenic hosts including piscivorous fish (Karmanova 1968, Measures 1988*d*), amphibians (Panesar and Beaver 1979), and reptiles (Lichtenfels and Lavies 1976, Goldberg et al. 1991). Spalding (pers. com.) reported

finding larvae of *Eustrongylides* in pig frogs (*Rana grylio*) and juvenile aquatic turtles from Florida. Cooper et al. (1978) transferred *E. tubifex* larvae from yellow perch (*Perca flavescens*)to grass frogs (*R. pipiens*), snapping turtles (*Chelydra serpentina*), Blandings turtle (*Emys blandingi*), and spiny soft-shelled turtle (*Trionyx spinifer*). Measures (1988*b*) infected fish paratenic hosts (*L. gibbosus*) with encapsulated fourth-stage *E. tubifex* larvae from naturally infected fish. Larvae were then transferred from paratenic hosts to birds (Measures 1988*d*). Although advanced-stage larvae of *Eustrongylides*, similar to those reported from fish, have been observed in several species of amphibians and reptiles, experimental transmission from these animals to birds was not done (Panesar and Beaver 1979, Lichtenfels and Lavies 1976, Goldberg et al. 1991). Fourth-stage larvae in all poikilotherms are probably infective to birds because of the similarity in morphology and behavior of larvae in fish, amphibians, and reptiles. In the present study, fourth-stage *E. ignotus* larvae were transferred from mosquitofish to large piscivorous fishes including black crappie, bluegill, largemouth bass, and warmouth. Both single and multiple infections were observed in large fishes. This

supports the accumulation hypothesis of Von Brand (1944), in which paratenic hosts may function as reservoirs for *E. ignotus* larvae with larger (and presumably older fishes) capable of supporting higher intensities of parasite larvae than smaller fishes. Larvae excysted and migrated into organs, muscle tissues, or the coelomic cavity. In paratenic hosts, most larvae were free, although some were encysted. Formation of cysts in paratenic hosts probably requires ≥ 30-days. The larvae remained in the fourth-stage of development in fish, which is infective to birds (Spalding et al. 1993).

In Florida, adult wading birds with large body sizes such as great blue herons and great egrets were reported to have higher prevalences of *E. ignotus* than birds with small body sizes such as green-backed herons (*Butorides virescens*) and snowy egrets (*Egretta thula*) (Spalding et al. 1993). Body size, age, and bill length of wading birds were shown to be positively correlated with prey size (Kushlan 1978). Likewise, the prevalence of eustrongylidosis in ardeid nestlings increased significantly with bill length and age (Spalding et al. 1993). Birds which prey on larger paratenic hosts may be at greater risk of contracting

eustrongylidosis because of bio-accumulation of the parasite in these fishes.

In addition to poikilotherms, larvae of *Eustrongylides* have been reported from fish-eating mammals (including humans), but these were probably abnormal hosts and not involved in the natural life cycle of the parasite (Gibson and McKiel 1972, Abram and Lichtenfels 1974, Eberhard et al. 1989). Most *Eustrongylides* infections, in paratenic and abnormal hosts, were probably acquired through ingestion of infected fish.

## Fish Behavior

Many parasitic helminths alter the behavior of hosts in ways that appear to increase the probability of parasite survival (Hurd 1990). Metacercaria of the liver fluke *Dicrocoelium dendriticum* paralyze of the mandibular muscles of formicine ants (intermediate hosts), causing the ants to remain attached to vegetation increasing the probability of accidental ingestion by grazing ungulates (definitive hosts) (Hohorst and Graefe 1961). *Acanthocephalus lucii* causes change in the pigmentation and behavior of aquatic isopod (intermediate hosts), which make them more susceptible to predation by perch (definitive host) (Brattey 1983). In the present study, intermediate and paratenic hosts were

observed in order to determine if infection with larval *E. ignotus* affected predator avoidance and host behavior.

Infected mosquitofish were selected over non-infected fish in 82% of trials. Although infected mosquitofish had a well defined abdominal mass (larval parasite encysted in coelomic cavity), it was unclear if physical appearance was a factor in prey selection. Movement of infected mosquitofish is the most plausible explanation for predator selection. Centrarcids are sight predators and cue on movement (Williams 1994). Although both infected and non-infected mosquitofish sought refuge in vegetation, infected fish appeared to exhibit frequent erratic movement. Coiled larvae often moved inside the cysts and may have caused discomfort and erratic movement in infected mosquitofish.

Whatever the mechanism, *E. ignotus* larvae produced an obvious burden on mosquitofish, which made infected fish more susceptible to predation. The mean number of capture attempts was 2.7 for infected fish and 3.9 for non-infected fish. In addition, predators captured infected prey in nearly half the time required for capture of non-infected fish. Holmes and Bethel (1972) examined predator-prey relationships and suggested 2 methods by which parasites

increased the likelihood of intermediate host predation including 1) reduction in host stamina and locomotor efficiency and 2) host disorientation. *Eustrongylides ignotus* is large relatively, when compared to mosquitofish body size, and probably produces a physical and metabolic burden which may reduce stamina and inhibit the fish's ability to avoid capture. Although not tested for in the present study, larvae may produce allomones (chemical compounds produced by one species to evoke physiological or behavioral reactions in another species, which are favorable to the first) (Brown et al. 1970).

Infected mosquitofish are probably more susceptible to predation by foraging wading birds also. Herons and egrets use many of the same visual cues to select prey as centrarchids (e.g. movement and prey exposure) (Kushlan 1978). Dence (1958) reported that shiners (*Notropis* spp.) infected with the parasite *Ligula intestinalis* were sluggish, less gregarious, and frequented shallow warm waters near shore more often than uninfected fish, even when avian predators were present. When fish did evade capture by retreating to deeper water, they returned to shore quickly. In another study of the same parasite, Holmes and

Bethel (1972) reported that 30% of fish in the stomach contents of great cormorants were infected, whereas only 6.5% of fish in the natural populations were infected. They concluded that parasitized fish were more susceptible to predation by piscivorous birds than non-parasitized fish.

Nestling wading birds, fed large numbers of mosquitofish may be at greater risk of infection with *Eustrongylides* than nestlings fed other prey items such as crustaceans and medium size fishes. Frederick and Collopy (1988) reported that small fishes (including mosquitofish and small centrarchids) made up a significant portion of the diet of nestling Snowy Egrets and Little Blue Herons in Florida, while crustaceans and medium size fishes were observed from nestling Great Blue Herons (Hoffman et al 1994). Although large fish may be paratenic hosts, some species of medium size fishes, selected by Great Blue Herons for their nestlings, may be too small to consume infected mosquitofish. These differences in prey selection may help explain the observations by Spalding et al. (1993), who reported higher prevalences of *E. ignotus* in Snowy Egret and Little Blue Heron nestlings than in Great Blue Heron nestlings from Florida. Most of the nestling Great Blue

Herons collected by Spalding et al. (1993) were from estuarine sites in Florida Bay (Spalding pers. comm.) which explain the low prevalence of infection also.

Under experimental conditions, aberrant behavior was observed in 67% of predatory fishes after ingestion of infected mosquitofish. These signs included lethargy, loss of appetite, convulsions, and buoyancy abnormalities. Fourth stage larvae penetrated the intestinal tract and migrated into the surrounding tissue shortly after ingestion. At necropsy, fourth-stage larvae were collected from various organs such as the liver, kidney, and air bladder. Aberrant behavior of the paratenic host depends opon which tissues are affected and the amount of damage done by migrating parasites. If the paratenic host survives initial parasite migration, the larva will encyst and may remain in the infective fourth-stage for a long period of time. In addition, predators may be re-infected each time an infected mosquitofish is ingested.

Adult wading birds that select for large fish with multiple *E. ignotus* infections are probably at greater risk than birds which forage on small fish. Solitary adult Great Blue Herons forage in deeper water and take significantly

larger prey than do smaller herons and egrets, which often forage in groups (Hoffman et al. 1994). This may help explain why Spalding et al. (1993) observed higher prevalences of *E. ignotus* in adult Great Blue Herons than in adults of smaller wading bird species.

## Life Cycle Summary

The life cycle of *E. ignotus* is complex, characterized by a number of different possible pathways, and variable in the amount of time required for completion (Fig. 20). Under experimental conditions, eggs required 17 to 22 days incubation before they were infective to intermediate hosts. Under natural conditions, this time period may be less.

Two pathways of parasite transmission to fish intermediate hosts were observed, including direct transmission through ingestion of infective eggs and indirect transmission through ingestion of infected aquatic oligochaetes. If mosquitofish ingested mature eggs, larvae developed to the infective L-4 in 84 to 105 days PI. The parasite was then infective to other fish paratenic hosts or bird definitive hosts. In wading birds, *E. ignotus* larvae mature to the adult-stage and begin producing eggs 14 to 23

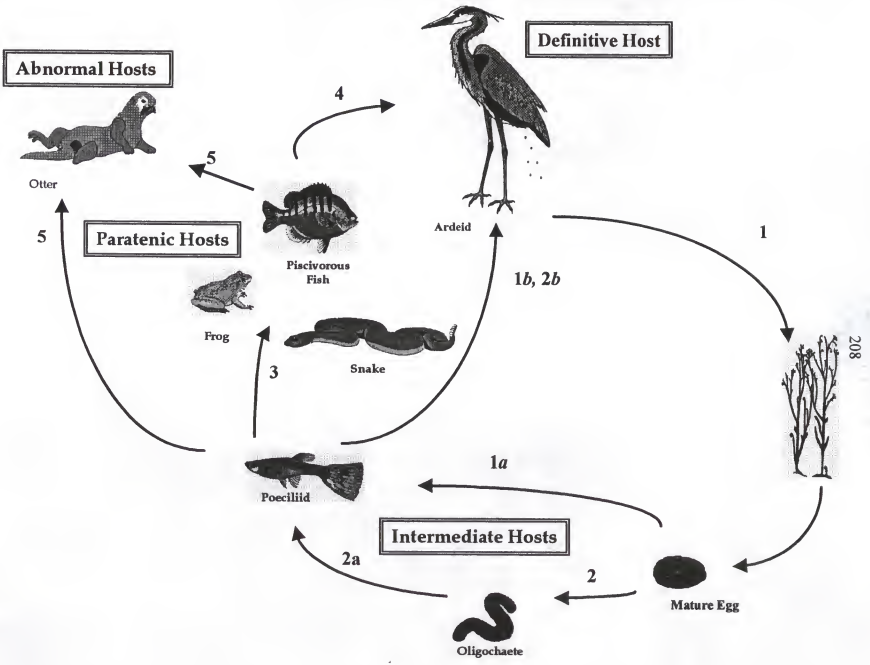
Abnormal Hosts
Definitive Host
Otter
4
5
5
Paratenic Hosts
Piscivorous Fish
Ardeid
1
1b, 2b
Frog
Snake
3
208
1a
Poeciliid
Intermediate Hosts
2a
2
Mature Egg
Oligochaete

Figure 20. The life cycle of *Eustrongylides ignotus* in Florida.

1. Eggs of *E. ignotus* are deposited into the water with bird feces. Eggs adhere to vegetation or fall to the sediment with detritus and develop to the infective-stage (L-1) in 17 to 22 days.

    1*a*. Egg is ingested by Eastern mosquitofish (*Gambusia holbrooki*). Larva develops to L-4 (infective to next host) in 84 to 105 days.
    1*b*. Mosquitofish is ingested by ardeid. Larva perforates the stomach, matures, mates, and produces eggs (17 to 22 days). Total time elapsed: 115 to 150 days.

2. Egg is ingested by oligochaete (*Limnodrillus* sp. or *L. hoffmeisteri*). Larva matures to L-3 (infective to fish) in ≥180 days.

    2*a*. Oligochaete is ingested by mosquitofish. Larva matures to L-4 in 127 - 184 days.
    2*b*. Mosquitofish is ingested by ardeid. Larva perforates the stomach, matures, mates, and produces eggs (17 to 22 days). Total time elapsed: 338 to 408 days.

3. Mosquitofish is ingested by piscivorous fish, amphibian, or reptile. Infected mosquitofish is more susceptible to predation than non-infected mosquitofish. Infected fish paratenic host may be more susceptible to predation by wading birds than non-infected fish. Larva remains infective to birds for ≥ 1 yr.

4. Paratenic host is ingested by ardeid. Total time: 115 days to ≥ 1 yr.

5. Mammal abnormal hosts (including humans) ingest larva in uncooked fish, amphibians, or reptiles. The parasite does not mature, but may cause severe lesions in individuals.

days PI (Spalding et al. 1994). Total time elapsed for this pathway is 115 to 150 days.

Aquatic oligochaetes (immature *Limnodrilus* sp. and *L. hoffmeisteri*) may act as primary intermediate hosts. This pathway is probably an adaptation to sites where the parasite must over-winter or regions where fish are not active during cold weather, such as in northern latitudes (Measures 1988*d*). Larvae in oligochaetes required at least 180-days incubation before they were infective to fish secondary intermediate hosts. Once ingested by fish, the larvae were infective to other fish paratenic hosts or bird definitive hosts at 127 to 184 days PI. Total time elapsed for this pathway is 338 to 408 days. Larvae of *Eustrongylides* probably develop slower in oligochaetes than in fish because of the small size and less nutritional resources available in oligochaetes than in fish. Once larvae develop in intermediate or paratenic hosts, they probably remain in the environment and are infective to wading birds for ≥ 1-yr.

Management Implications

*Eustrongylides ignotus* has probably been cycling in the Florida environment for a long period of time, although at low prevalences. Historical reports of *Eustrongylides* in fishes go back to the 1930's, but the parasite was probably present long before it was first observed. Outbreaks of eustrongylidosis appear to be associated with long-term human activities such as nutrient input, habitat disturbance by excavation or dredging, and stabilization of hydroperiod. Long-term anthropogenic nutrient input such as the discharge of sewage effluent or urban runoff may produce eutrophic conditions, with low dissolved oxygen and high densities of potential intermediate hosts. Excavation or dredging increases erosion, disturbs the vegetation and sediment, and probably results in increased amounts of benthic silt and clay in the sediment of enzootic sites. Stabilization of hydroperiod (especially reduction of drydown periods) may help maintain enzootic sites. The parasite is vulnerable to dehydration from the egg-stage through ingestion by the definitive host. Eggs are susceptible to desiccation and most intermediate hosts require aquatic habitat for survival. I observed that enzootic sites were localized in

small human-disturbed habitats, although these sites were distributed widely throughout the state. Breeding wading birds that forage in these types of impacted sites may be at risk. As the human population of Florida increases and urban areas expand, the number of wetland habitats suitable for outbreaks of eustrongylidosis in wading birds will probably increase also.

Agencies responsible for monitoring and managing surface waters should be informed of this potential wildlife disease problem and taught to recognize the parasite in fish intermediate hosts. Periodic monitoring of fish from human altered sites with large numbers of foraging wading birds may be recommended, especially prior to breeding season. If high prevalences of infected fishes are detected, this disease problem can probably be mitigated in some cases by allowing drydown of the enzootic site, thus removing potential intermediate hosts and desiccation of parasite eggs.

In human constructed impoundments and artificial wetlands receiving large amounts of nutrients, managers may be encouraged to maintain dissolved oxygen concentrations $\geq$ 5 mg/l through artificial aeration or natural design. Fish

populations should be balanced with large predators to help regulate poeciliid densities.

Although probably impossible to eradicate completely, agencies and the public should be aware of the potential impact of this parasite on breeding wading bird populations and its association with human habitat alteration.

## CONCLUSIONS

The purpose of this section is to address briefly each of our major objectives and summarize the observations of the study. This will be done by listing the objectives in order and giving a brief summation of findings for each as follows:

1). Determine which oligochaete worms, or other aquatic invertebrates, are primary intermediate hosts for the parasite.

Under laboratory conditions, *E. ignotus* developed in the aquatic oligochaete *Limnodrilus hoffmeisteri* and in immature tubificids which were identified as *Limnodrillus* sp. The parasite did not develop in other common oligochaetes or chironomids.

2). Identify which fishes in Florida serve as intermediate hosts of *Eustrongylides*. In addition, determine the

distribution in Florida of intermediate hosts (fishes and oligochaetes) infected with larvae of *Eustrongylides*.

In total, *Eustrongylides* has now been reported from 16 species of fishes in Florida. It is obvious the parasite is not restricted to fishes of any specific taxonomic group. Feeding behavior may explain the broad range of infected species. In this study, when infected fish from positive sites in Florida were separated into the broad categories of predators (Centrarchidae and Lepisosteidae) and prey (Cyprinodontidae and Poeciliidae), the prevalence of *Eustrongylides* was higher in predators (4.5%) than prey (1.8%). Based on the percent of parasites collected from infected predatory fish in Florida, Centrarchidae (sunfishes) were the most important paratenic hosts, while Poeciliidae (livebearers) were the most important intermediate hosts.

Fishes, infected with larvae of *Eustrongylides*, were collected from sites receiving human sewage, urban, or agriculture runoff. Infected fishes were not found at natural sites. Throughout peninsular Florida, the percent

of sites with infected fishes was highest in northern Florida, followed by central and southern Florida.

Under experimental conditions, *Limnodrilus* sp. and *L. hoffmeisteri* were infected with *Eustrongylides*. Many oligochaetes of the genus *Limnodrilus* are widely distributed and locally abundant in eutrophic Florida waters. The density of *L. hoffmeisteri* was greater at positive than negative sites.

3). Determine the environmental conditions necessary for the production of large numbers of primary intermediate hosts (oligochaetes) and infected secondary intermediate hosts (fish).

All positive sites were characterized by human alterations such as excavation or dredging and had some obvious source of exogenous nutrient input. Positive sites had lower dissolved oxygen and higher concentrations of total nitrogen, total phosphorus, and chlorophyll-*a* than negative sites. Mean sediment grain size was larger at positive sites and these sites also had higher percent of silt and clay also. Vegetation was dominated by emergents

and grasses and algae blooms were often observed at positive sites.

4). Determine how long parasite eggs take to become infectious to oligochaete worms after being excreted by birds.

Under experimental conditions and depending on water temperature, *E. ignotus* eggs developed in 17 - 21-days and were infective to mosquitofish and oligochaetes. Under natural conditions, the time required for development to the infective-stage may be less. The eggs were vulnerable to desiccation during this time and did not develop in seawater, but did develop in brackish water. During this time, eggs are very sticky and probably adhere to vegetation and detritus throughout the water column, where they are consumed by intermediate hosts.

5). Determine the time it takes for the parasite to mature to an infective stage in fishes, once ingested.

Fishes were primary or secondary intermediate hosts and paratenic hosts. If mosquitofish were primary intermediate hosts, the parasite required ≥ 125-days incubation to develop to the fourth-stage larva. If mosquitofish were secondary intermediate hosts and ingested infected oligochaetes, 127 - 184-days were necessary for development. Once larvae developed to the fourth-stage, they were infective to piscivorous fish. Larvae survived in the fourth-stage (infective to birds) for at least several months and could possibly survive for the life of the fish.

6). Determine the role of other organisms, such as turtles, frogs, and snakes as possible secondary intermediate hosts at sites with infected fish.

I examined turtles, frogs, and snakes from positive sites but none was infected with larvae of *Eustrongylides*, although Spalding (pers. com.) has observed larvae of *Eustrongylides* in pig frogs and turtles from Florida. Piscivorous amphibians and reptiles may be paratenic hosts, but the distribution of infected individuals appears to be

patchy and probably the result of the high prevalences of infected fish at the site.

7). Determine behavioral changes in infected fish.

I determined that infected mosquitofish were more susceptible to predation by larger predatory hosts than non-infected mosquitofish. Infected mosquitofish were selected first, required fewer capture attempts, and were captured in less time than non-infected fish. Predatory fish were observed for 4-days post-ingestion and 67% showed signs of aberrant behavior such as lethargy, convulsions, loss of appetite, and buoyancy abnormalities. Although not tested, infected fish intermediate hosts and paratenic hosts may be more susceptible to predation by wading birds than non-infected fish.

# APPENDIX
# LOCATIONS OF STUDY SITES

Table A. Locations of study sites in peninsular Florida, 1994-1997. Sites are designated positive or negative for *Eustrongylides ignotus* larvae in fish. Site types are: A=agricultural, N=natural, S=sewage, U=urban. Sites are sorted by region, parasite presence, and site number.

| SITE No. | SITE NAME | PARASITE | COUNTY | TYPE | REGION | LAT/LONG |
|---|---|---|---|---|---|---|
| 1 | UFTP-A | Pos | Alachua | U | North | N 29° 38.547' W 82° 20.680' |
| 3 | UFTP-B | Pos | Alachua | S | North | N 29° 38.450' W 82° 20.978' |
| 4 | UFTP-B.5 | Pos | Alachua | S | North | N 29° 38.451' W 82° 20.985' |
| 5 | UFTP-C | Pos | Alachua | S | North | N 29° 38.449' W 82° 21.119' |
| 6 | Hogtown-Univ. | Pos | Alachua | U | North | N 29° 39.171' W 82° 22.318' |
| 8 | UF Dairy | Pos | Alachua | A | North | N 29° 37.488' W 82° 21.334' |
| 9 | SEA-Grille | Pos | Alachua | U | North | N 29° 38.457' W 82° 18.464' |
| 10 | Newnans BR | Pos | Alachua | U | North | N 29° 36.404' W 82° 14.542' |
| 16 | PP site 2 | Pos | Alachua | S | North | N 29° 37.443' W 82° 19.166' |
| 18 | PP site 4 | Pos | Alachua | S | North | N 29° 36.515' W 82° 19.168' |
| 21 | Northwood | Pos | Alachua | U | North | N 29° 42.234' W 82° 21.431' |
| 142 | NW 69th Ter. | Pos | Alachua | U | North | N 29° 39.433' W 82° 24.591' |
| 144 | NW 69th Ter. South | Pos | Alachua | U | North | N 29° 39.441' W 82° 24.562' |
| 165 | River Styx | Pos | Alachua | U | North | N 29° 31.040' W 82° 13.025' |
| 2 | UFTP-A.5 | Neg | Alachua | U | North | N 29° 38.426' W 82° 20.851' |
| 7 | Hogtown 2ave | Neg | Alachua | U | North | N 29° 39.400' W 82° 22.318 |
| 11 | Hague Pond | Neg | Alachua | A | North | N 29° 46.270' W 82° 25.345' |
| 12 | Hague Stream | Neg | Alachua | A | North | N 29° 46.105' W 82° 25.345' |
| 13 | Watermelon Pond | Neg | Alachua | N | North | N 29° 33.323' W 82° 36.390' |
| 14 | Lake Mize | Neg | Alachua | N | North | N 29° 43.558' W 82° 13.109' |
| 15 | SB rd321 | Neg | Alachua | S | North | N 29° 37.484' W 82° 19.199' |
| 17 | PP site 3 | Neg | Alachua | S | North | N 29° 36.546' W 82° 19.311' |
| 19 | PP site 5 | Neg | Alachua | S | North | N 29° 36.518' W 82° 19.117' |
| 20 | PP Lake | Neg | Alachua | N | North | N 29° 36.543' W 82° 19.070' |
| 24 | Hawthorne BR | Neg | Marion | U | North | N 29° 35.279' W 82° 05.002' |
| 92 | UFTP-D | Neg | Alachua | N | North | N 29° 38.530' W 82° 21.811' |
| 110 | Lake Wauberg | Neg | Alachua | N | North | N 29° 32.078' W 82° 18.128' |
| 111 | Bivens Arm | Neg | Alachua | U | North | N 29° 37.178' W 82° 20.201' |
| 112 | Bellamy | Neg | Alachua | U | North | N 29° 40.051' W 82° 23.297' |
| 143 | NW 69th Ter. Beta | Neg | Alachua | U | North | N 29° 39.418' W 82° 24.568' |
| 166 | Santa Fe | Neg | Alachua | U | North | N 29° 40.559' W 82° 26.071' |
| 168 | Cross Creek BR | Neg | Alachua | N | North | N 29° 28.087' W 82° 09.544' |
| 170 | Marshal Pond | Neg | Putnam | A | North | N 29° 36.300' W 82° 09.076' |

Table A. Continued.

| SITE No. | SITE NAME | PARASITE | COUNTY | TYPE | REGION | LAT/LONG |
|---|---|---|---|---|---|---|
| 145 | Apopka I | Pos | Orange | A | Central | N 28° 38.388' W 81° 33.523' |
| 150 | Apopka VI | Pos | Lake | U | Central | N 28° 33.567' W 81° 33.912' |
| 156 | Hancock I | Pos | Polk | U | Central | N 27° 59.550' W 81° 51.119' |
| 157 | Hancock II | Pos | Polk | S | Central | N 27° 58.223' W 81° 51.090' |
| 181 | Hancock III | Pos | Polk | A | Central | N 27° 58.211' W 81° 51.297' |
| 22 | Thanotossasa | Neg | Hillsborough | N | Central | N 28° 03.039' W 82° 16.354' |
| 23 | Baker Creek | Neg | Hillsborough | S | Central | N 28° 02.533' W 82° 16.050' |
| 25 | V Gardens | Neg | Lake | U | Central | N 28° 48.169' W 81° 52.295' |
| 26 | Lake Yale | Neg | Lake | A | Central | N 28° 55.182' W 81° 52.295' |
| 27 | Orl R.I.B. | Neg | Orange | S | Central | N 28° 26.287' W 81° 24.216' |
| 28 | Orl exit | Neg | Orange | S | Central | N 28° 31.194' W 81° 23.410' |
| 29 | Orl-Redistr | Neg | Orange | S | Central | N 28° 26.230' W 81° 24.216' |
| 93 | Lees TP | Neg | Lake | S | Central | N 28° 48.513' W 81° 52.510' |
| 94 | Lees-River | Neg | Lake | S | Central | N 28° 48.515' W 81° 52.604' |
| 95 | Lees-BR | Neg | Lake | S | Central | N 28° 48.514' W 81° 52.580' |
| 146 | Apopka II | Neg | Orange | A | Central | N 28° 38.259' W 81° 33.189' |
| 147 | Apopka III | Neg | Orange | A | Central | N 28° 37.928 W 81° 33.070' |
| 148 | Apopka IV | Neg | Orange | A | Central | N 28° 37.109' W 81° 33.657' |
| 149 | Apopka V | Neg | Orange | U | Central | N 28° 34.590' W 81° 35.311' |
| 151 | Banana I | Neg | Lake | A | Central | N 27° 58.862' W 81° 54.513' |
| 152 | Banana II | Neg | Lake | N | Central | N 27° 58.896' W 81° 53.743' |
| 153 | Banana III | Neg | Lake | A | Central | N 27° 58.904' W 81° 53.744' |
| 154 | Banana IV | Neg | Lake | N | Central | N 27° 58.662' W 81° 53.755' |
| 155 | Banana V | Neg | Lake | N | Central | N 27° 58.501' W 81° 54.187' |
| 158 | Hancock Marsh | Neg | Polk | N | Central | N 27° 58.198' W 81° 51.305' |
| 172 | Marsh Pond | Neg | Lake | N | Central | N 28° 49.226' W 81° 50.149' |
| 173 | Emerald Marsh I | Neg | Lake | A | Central | N 28° 55.134' W 81° 46.272' |
| 174 | Emerald Marsh II | Neg | Lake | A | Central | N 28° 54.499' W 81° 47.279' |
| 175 | Emerald Marsh III | Neg | Lake | A | Central | N 28° 54.129' W 81° 49.367' |
| 176 | Emerald Marsh IV | Neg | Lake | A | Central | N 28° 55.178' W 81° 48.225' |
| 182 | Hancock IV | Neg | Polk | N | Central | N 27° 57.304' W 81° 51.290' |
| 183 | Apopka VII | Neg | Lake | U | Central | N 28° 33.293' W 81° 38.512' |
| 184 | Hancock Springs | Neg | Polk | N | Central | N 27° 59.551' W 81° 50.197' |

Table A. Continued.

| SITE No. | SITE NAME | PARASITE | COUNTY | TYPE | REGION | LAT/LONG |
|---|---|---|---|---|---|---|
| 31 | S.839BR | Pos | Collier | U | South | N 25° 53.132' W 81° 15.693' |
| 33 | Loop rd S1 | Pos | Monroe | U | South | N 25° 45.631' W 80° 51.734' |
| 38 | Monroe St. | Pos | Collier | U | South | N 25° 51.796' W 81° 06.027' |
| 52 | Lox S-6D | Pos | Palm Beach | A | South | N 26° 28.211' W 80° 28.170' |
| 81 | MF-SP | Pos | Okeechobee | A | South | N 27° 26.182' W 80° 48.389' |
| 82 | MF-TP | Pos | Okeechobee | A | South | N 27° 26.182' W 80° 47.363' |
| 89 | site 3-south | Pos | St. Lucie | A | South | N 27° 16.714' W 80° 21.110' |
| 99 | Holiday Park I | Pos | Dade | U | South | N 26° 03.640' W 80° 26.529' |
| 140 | S-5A BR | Pos | Palm Beach | A | South | N 26° 40.980' W 80° 22.253' |
| 191 | Jog Rd Alpha | Pos | Palm Beach | S | South | N 26° 35.432' W 80° 08.531' |
| 192 | Jog Rd Beta | Pos | Palm Beach | S | South | N 26° 35.430' W 80° 09.011' |
| 30 | 839 N | Neg | Collier | N | South | N 25° 54.025' W 81° 15.734' |
| 32 | Loop rd S2 | Neg | Monroe | N | South | N 25° 44.794' W 80° 57.233' |
| 34 | Birdon Rd | Neg | Collier | N | South | N 25° 54.016' W 81° 15.715' |
| 35 | Tower BR | Neg | Dade | N | South | N 25° 45.776' W 80° 49.166' |
| 36 | Dade FTS | Neg | Dade | U | South | N 25° 52.086' W 80° 53.964' |
| 37 | 837 W | Neg | Collier | N | South | N 25° 59.649' W 81° 18.041' |
| 40 | IMM TP | Neg | Collier | S | South | N 26° 25.158' W 81° 27.322' |
| 41 | TYL-1 | Neg | Okeechobee | U | South | N 27° 13.240' W 80° 48.006' |
| 42 | BHR-1 | Neg | Glades | N | South | N 27° 07.201' W 80° 53.517' |
| 43 | DLD WWRP | Neg | Okeechobee | U | South | N 27° 18.408' W 80° 54.076' |
| 44 | DLD #1 | Neg | Okeechobee | U | South | N 27° 18.372' W 80° 54.068' |
| 45 | Okee Dairy | Neg | Okeechobee | A | South | N 27° 27.410' W 80° 56.292' |
| 46 | Shark END | Neg | Monroe | N | South | N 25° 24.522' W 81° 00.259' |
| 47 | HW Ruck Dairy | Neg | Okeechobee | A | South | N 27° 25.077' W 80° 51.365' |
| 48 | PBC SWL #3 | Neg | Palm Beach | U | South | N 26° 19.520' W 80° 12.174' |
| 49 | Shark END-2 | Neg | Monroe | N | South | N 25° 23.194' W 81° 00.436' |
| 50 | Shark END-3 | Neg | Monroe | N | South | N 25° 22.542' W 81° 01.521' |
| 51 | Flamingo EP | Neg | Monroe | N | South | N 25° 08.178' W 80° 56.200' |
| 53 | Lox #S-5 | Neg | Palm Beach | A | South | N 26° 41.030' W 80° 21.520' |
| 54 | Lox #1 | Neg | Palm Beach | N | South | N 26° 36.048' W 80° 16.449' |
| 55 | Lox S-5A | Neg | Palm Beach | A | South | N 26° 41.060' W 80° 21.517' |
| 56 | Lox S-6 UP | Neg | Palm Beach | N | South | N 26° 28.211' W 80° 26.415' |
| 57 | Lox S-6 | Neg | Palm Beach | A | South | N 26° 28.177' W 80° 26.435' |
| 58 | Lox S-5 A side | Neg | Palm Beach | U | South | N 26° 41.438' W 80° 21.509' |
| 59 | Lox B-2 | Neg | Palm Beach | N | South | N 26° 31.340' W 80° 13.351' |

Table A. Continued.

| SITE No. | SITE NAME | PARASITE | COUNTY | TYPE | REGION | LAT/LONG |
|---|---|---|---|---|---|---|
| 60 | Lox #2 | Neg | Palm Beach | N | South | N 26° 30.539' W 80° 13.131' |
| 61 | WCA3 site 3 | Neg | Broward | N | South | N 25° 54.200' W 80° 35.090' |
| 62 | St. L West | Neg | St. Lucie | S | South | N 27° 16.226' W 80° 21.166' |
| 63 | Treasure 1 | Neg | Glades | U | South | N 27° 10.536' W 80° 55.118' |
| 64 | Treasure 2 | Neg | Glades | U | South | N 27° 10.121' W 80° 54.547' |
| 65 | NLO Taylor | Neg | Okeechobee | U | South | N 27° 21.390' W 80° 39.440' |
| 66 | Century 1 | Neg | Palm Beach | U | South | N 26° 42.482' W 80° 07.385' |
| 67 | Century 2 | Neg | Palm Beach | U | South | N 26° 42.533' W 80° 07.291' |
| 68 | St. L small c | Neg | St. Lucie | U | South | N 27° 16.081' W 80° 20.117' |
| 69 | Holeyland | Neg | Palm Beach | N | South | N 26° 15.150' W 80° 44.480' |
| 70 | Jac WCD | Neg | Broward | U | South | N 26° 12.251' W 80° 16.385' |
| 72 | WP-Alpha | Neg | Palm Beach | S | South | N 26° 32.596' W 80° 08.844' |
| 73 | PBC LF#1 | Neg | Palm Beach | U | South | N 26° 46.317' W 80° 08.412' |
| 74 | PBC LF#2 | Neg | Palm Beach | U | South | N 26° 46.505' W 80° 09.148' |
| 75 | PBC Rookery | Neg | Palm Beach | U | South | N 26° 47.385' W 80° 09.043' |
| 76 | PBC N Rookery | Neg | Palm Beach | U | South | N 26° 47.210' W 80° 08.354' |
| 77 | PBC SWL #4 | Neg | Palm Beach | U | South | N 26° 45.353' W 80° 08.328' |
| 78 | PBC 1D | Neg | Palm Beach | U | South | N 26° 45.353' W 80° 09.091' |
| 79 | CL-1 | Neg | Palm Beach | U | South | N 29° 03.323' W 81° 15.314' |
| 80 | CL-2 | Neg | Palm Beach | U | South | N 29° 03.394' W 81° 15.397' |
| 83 | MF-#2 | Neg | Okeechobee | A | South | N 27° 26.180' W 80° 47.360' |
| 84 | Rucks | Neg | Okeechobee | A | South | N 27° 24.536' W 80° 51.216' |
| 85 | Mattison | Neg | Okeechobee | A | South | N 27° 27.389' W 80° 56.285' |
| 86 | WCA3 site 2 | Neg | Broward | N | South | N 25° 57.520' W 80° 32.420' |
| 87 | site 1-south | Neg | St. Lucie | U | South | N 27° 17.725' W 80° 21.182' |
| 88 | site 2-south | Neg | St. Lucie | U | South | N 27° 17.618' W 80° 20.118' |
| 90 | site 4-south | Neg | St. Lucie | U | South | N 27° 16.810' W 80° 21.121' |
| 91 | Field Gate | Neg | Dade | U | South | N 25° 50.763' W 80° 55.524' |
| 96 | Tam. East | Neg | Dade | N | South | N 25° 45.271' W 80° 31.398' |
| 97 | Lox SWC | Neg | Palm Beach | N | South | N 26° 28.220' W 80° 26.401' |
| 98 | Big Pond | Neg | Broward | N | South | N 26° 00.467' W 80° 26.058' |
| 100 | Hdq Pond | Neg | Palm Beach | U | South | N 26° 28.547' W 80° 12.504' |
| 101 | Loop rd S3 | Neg | Monroe | N | South | N 25° 45.607' W 81° 02.149' |
| 102 | L-28 | Neg | Collier | N | South | N 25° 47.207' W 80° 51.271' |
| 103 | Gap Tower Plug | Neg | Collier | N | South | N 28° 45.530' W 80° 33.415' |
| 104 | Lox SWC BR | Neg | Palm Beach | A | South | N 26° 28.310' W 80° 26.434' |

Table A. Continued.

| SITE No. | SITE NAME | PARASITE | COUNTY | TYPE | REGION | LAT/LONG |
|---|---|---|---|---|---|---|
| 105 | L-28 intercept | Neg | Broward | N | South | N 25° 59.262' W 80° 50.206' |
| 106 | south of intercept | Neg | Broward | N | South | N 25° 59.229' W 80° 50.162' |
| 107 | L-28 Tyback | Neg | Broward | N | South | N 25° 59.521' W 80° 51.534' |
| 108 | Robs Gator Hole | Neg | Broward | N | South | N 25° 45.494' W 80° 35.561' |
| 109 | Robs Reg... | Neg | Broward | N | South | N 25° 45.578' W 80° 35.538' |
| 113 | L-9 LNWR | Neg | Palm Beach | N | South | N 26° 27.779' W 80° 17.551' |
| 114 | L-9N | Neg | Palm Beach | N | South | N 26° 27.720' W 80° 17.385' |
| 116 | Lox BR | Neg | Palm Beach | N | South | N 26° 29.901' W 80° 13.318' |
| 117 | ENRP 1-A | Neg | Palm Beach | A | South | N 26° 39.387' W 80° 24.263' |
| 119 | ENRP 1-B | Neg | Palm Beach | A | South | N 26° 37.656' W 80° 25.912' |
| 121 | ENRP 2-A | Neg | Palm Beach | A | South | N 26° 38.930' W 80° 25.833' |
| 123 | ENRP 2-B | Neg | Palm Beach | A | South | N 26° 38.181' W 80° 25.000' |
| 125 | ENRP 4-A | Neg | Palm Beach | A | South | N 26° 38.094' W 80° 25.829' |
| 127 | ENRP 4-B | Neg | Palm Beach | A | South | N 26° 37.415' W 80° 26.384' |
| 130 | ENRP 3-A | Neg | Palm Beach | A | South | N 26° 37.641' W 80° 26.129' |
| 132 | ENRP 3-B | Neg | Palm Beach | A | South | N 26° 37.105' W 80° 26.222' |
| 134 | ENRP 3-C | Neg | Palm Beach | A | South | N 26° 36.764' W 80° 26.255' |
| 136 | ENRP 3-D | Neg | Palm Beach | A | South | N 26° 36.799' W 80° 26'.145' |
| 138 | ENRP 3-E | Neg | Palm Beach | A | South | N 26° 36.928' W 80° 26.729' |
| 160 | Lox BR' | Neg | Palm Beach | N | South | N 26° 29.820' W 80° 13.298' |
| 161 | Buffer Cell | Neg | Palm Beach | A | South | N 26° 39.385' W 80° 24.266' |
| 162 | Buffer Cell' | Neg | Palm Beach | A | South | N 26° 39.392' W 80° 24.380' |
| 163 | ENRP 2-C | Neg | Palm Beach | A | South | N 26° 38.593' W 80° 26.665' |
| 164 | ENRP 2-C' | Neg | Palm Beach | A | South | N 26° 38.498' W 80° 26.671' |
| 169 | MLD | Neg | Palm Beach | N | South | N 26° 27.725' W 80° 17.387' |
| 177 | ENRP Outflow | Neg | Palm Beach | A | South | N 26° 35.896' W 80° 26.605' |
| 178 | ENRP Outflow' | Neg | Palm Beach | A | South | N 26° 35.902' W 80° 26.712' |
| 180 | Bear Is. | Neg | Collier | N | South | N 26° 04.822' W 81° 15.879' |
| 185 | L-9 Alt | Neg | Palm Beach | N | South | N 26° 27.839' W 80° 17.891' |
| 187 | Lox G-250 | Neg | Palm Beach | N | South | N 26° 36.801' W 80° 24.382' |
| 189 | S-334 | Neg | Collier | N | South | N 26° 00.147' W 80° 25.940' |
| 190 | Holiday Park 11 | Neg | Dade | U | South | N 26° 03.652' W 80° 26.529' |
| 193 | HP WIlliams South | Neg | Collier | U | South | N 25° 36.385' W 82° 14.541' |
| 195 | Coconuts1l1D | Neg | Monroe | U | South | N 25° 44.010' W 81° 03.021' |
| 196 | Coconuts1l1L | Neg | Monroe | U | South | N 25° 44.051' W 81° 03.032' |

Table B. Locations of study sites in peninsular Florida, 1994-1997 and specimens collected. Sites are designated positive or negative for larvae of *Eustrongylides ignotus* in fish. Site types are: A=agricultural, N=natural, S=sewage, U=urban.

| SITE No. | PARASITE | REGION | TYPE | FISH DENSITY | OLIGOCHAETES | AMPHIBIANS REPTILES | WATER/ SEDIMENT | SOD | GRAIN SIZE | VEGETATION |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Pos | North | U | X | X | X | X | X | X | X |
| 3 | Pos | North | S | X | X | X | X | X | X | X |
| 4 | Pos | North | S | X | | | | | | |
| 5 | Pos | North | S | X | X | X | X | X | X | X |
| 6 | Pos | North | U | X | X | | X | X | X | X |
| 8 | Pos | North | A | X | X | | X | X | X | X |
| 9 | Pos | North | U | X | X | | | X | X | X |
| 10 | Pos | North | U | X | X | X | X | X | X | X |
| 16 | Pos | North | S | X | | | | | | |
| 18 | Pos | North | S | X | X | | X | X | X | X |
| 21 | Pos | North | U | X | X | X | X | X | X | X |
| 142 | Pos | North | U | X | X | | | | | |
| 144 | Pos | North | U | X | | X | X | X | X | |
| 165 | Pos | North | U | X | X | X | | X | X | X |
| 2 | Neg | North | U | | | | | | | |
| 7 | Neg | North | U | | | | X | X | X | X |
| 11 | Neg | North | A | X | X | | X | X | X | X |

| SITE No. | PARASITE | REGION | TYPE | FISH DENSITY | OLIGOCHAETES | AMPHIBIANS REPTILES | WATER / SEDIMENT | SOD | GRAIN SIZE | VEGETATION |
|---|---|---|---|---|---|---|---|---|---|---|
| 12 | Neg | North | A | X | X | | | | | |
| 13 | Neg | North | N | X | X | | X | X | X | X |
| 14 | Neg | North | N | X | X | | X | X | X | X |
| 17 | Neg | North | S | X | X | | X | | | |
| 20 | Neg | North | N | X | X | | | | | |
| 24 | Neg | North | U | X | X | | X | | | X |
| 92 | Neg | North | N | X | X | | X | X | X | X |
| 110 | Neg | North | N | X | X | | X | | X | X |
| 111 | Neg | North | U | X | X | | X | | X | X |
| 112 | Neg | North | U | X | | | | | | |
| 145 | Pos | Central | A | X | X | | X | X | X | X |
| 150 | Pos | Central | U | X | X | | X | X | X | X |
| 156 | Pos | Central | U | X | X | X | X | X | X | X |
| 157 | Pos | Central | S | X | X | X | X | X | X | X |
| 181 | Pos | Central | A | X | X | | | | | X |
| 22 | Neg | Central | N | X | X | | X | X | X | X |
| 23 | Neg | Central | S | X | X | | X | X | X | X |
| 25 | Neg | Central | U | X | X | | X | X | X | X |
| 26 | Neg | Central | A | X | X | | X | X | X | X |
| 93 | Neg | Central | S | X | X | | | X | X | X |
| 94 | Neg | Central | S | X | | | | | | |
| 95 | Neg | Central | S | X | | | | | | |

| SITE No. | PARASITE | REGION | TYPE | FISH DENSITY | OLIGOCHAETES | AMPHIBIANS REPTILES | WATER / SEDIMENT | SOD | GRAIN SIZE | VEGETATION |
|---|---|---|---|---|---|---|---|---|---|---|
| 146 | Neg | Central | A | X | X | | | X | X | X |
| 147 | Neg | Central | A | X | X | | | | | |
| 151 | Neg | Central | A | X | X | | | X | X | X |
| 152 | Neg | Central | N | X | X | | | | | |
| 172 | Neg | Central | N | X | X | | | | | |
| 173 | Neg | Central | A | X | X | | | | | |
| 182 | Neg | Central | N | X | X | | | X | X | X |
| 183 | Neg | Central | U | X | | | | | | |
| 33 | Pos | South | U | X | X | X | X | X | X | X |
| 38 | Pos | South | U | X | X | | X | X | X | X |
| 52 | Pos | South | A | X | X | | X | X | X | X |
| 81 | Pos | South | A | X | | | | X | X | X |
| 82 | Pos | South | A | X | | | | X | X | X |
| 89 | Pos | South | A | X | | | | | | |
| 99 | Pos | South | U | X | X | X | X | X | X | X |
| 140 | Pos | South | A | X | X | | X | X | X | X |
| 191 | Pos | South | S | X | X | | X | X | X | X |
| 192 | Pos | South | S | X | | | | | | X |
| 30 | Neg | South | N | X | X | | | | | |
| 32 | Neg | South | N | X | X | | X | X | X | X |
| 34 | Neg | South | N | X | X | | | | | |
| 40 | Neg | South | S | X | X | | X | | X | X |

| SITE No. | PARASITE | REGION | TYPE | FISH DENSITY | OLIGOCHAETES | AMPHIBIANS REPTILES | WATER / SEDIMENT | SOD | GRAIN SIZE | VEGETATION |
|---|---|---|---|---|---|---|---|---|---|---|
| 41 | Neg | South | U | X | X | | X | | | |
| 45 | Neg | South | A | X | X | | X | X | | |
| 53 | Neg | South | A | X | X | | X | X | X | X |
| 63 | Neg | South | U | X | X | | X | X | | |
| 117 | Neg | South | A | X | X | | | X | X | X |
| 121 | Neg | South | A | X | X | | | X | X | X |
| 125 | Neg | South | A | X | X | | | X | X | X |
| 130 | Neg | South | A | X | X | | | X | X | X |
| 190 | Neg | South | U | X | X | | | X | X | X |
| 193 | Neg | South | U | X | X | | | X | X | X |

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